Research Article

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Association among stress, salivary cortisol levels, and chronic periodontitis

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Purpose: Chronic periodontitis (CP) seems to be associated with stress and depression, but little information on this possible association is available in the literature. Thus, the objective of this study was to evaluate the association among stress, the salivary cortisol level (SCL), and CP.

Methods: Seventy systemically healthy subjects were included in the study from January to September 2011. Full medical and dental histories were obtained, and the following measurements were recorded: 1) probing depth; 2) clinical attachment level; 3) bleeding on probing; and 4) tooth mobility. Saliva samples were collected for the evaluation of SCL (via a highly sensitive electrochemiluminescence immunoassay), and all subjects also answered a questionnaire (i.e., the Zung Self-rating Depression Scale). The odds ratio (OR) with a 95% confidence interval (CI) was calculated, and one way analysis of variance and the Tukey-Kramer method were performed.

Results: A total of 36 subjects with CP (51.4%) and 34 without CP were evaluated. Of them, all of the subjects with CP and one periodontally healthy subject were diagnosed with depression. Subjects with moderate CP had statistically significantly higher levels of SCL than subjects with a diagnosis of slight CP (P=0.006). Also, subjects with severe CP showed the same outcome when compared to those with slight CP (P=0.012). In addition, 46 subjects presented high SCL whereas 24 had a normal level. CP was found to be correlated with the SCL, with an OR of 4.14 (95% CI, 1.43 to 12.01).

Conclusions: Subjects with a high SCL and depression may show an increased risk for CP.

Keywords: Chronic periodontitis, Depressive disorder, Physiological stress, Saliva.

INTRODUCTION

Plaque-induced chronic periodontitis (CP) is a multifactorial disorder where microbial dental biofilms are considered the main etiological agent for the initiation of the inflammation process [1-4]. Typically, the amount of periodontal loss produced by the disease is consistent with the presence of biofilm and the rate of progression is slow to moderate, but periods of rapid progression can occur [1,2,5-7].

Nevertheless, the progression and severity of disease may be associated with assorted conditions, especially when a patient is exposed to one or more risk factors known to influence the host response [2-4]. It has been reported that local, systemic, or environmental factors (e.g., race-ethnicity, socioeconomic status, oral hygiene level, diabetes mellitus, or smoking) can contribute to the amount of periodontal tissue loss [3,4,8]. For instance, smokers are at a higher risk of more severe marginal bone loss over time when compared with

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never smokers [9].

With respect to other possible factors influencing CP, evidence is emerging that chronic stress (CS) and depression and anxiety may negatively influence disease progression [10-17]. CS is a recognized risk factor that affects diverse systemic conditions and diseases [18-20], probably due its stimulation of the hypothalamus-pituitary-adrenal (HPA) cortex axis [16,17,21]. Once stimulated, this area leads to an increase of blood and saliva cortisol levels (SCL) [18-20]. Cortisol is a hormone related to stress produced by the adrenal gland, and causes a series of events in the human body, such as the formation of glucose and activation of antistress and anti-inflammatory mechanisms [18-20].

It has been argued that the relationship between stress and periodontitis seems to be linked to the lack of regular oral hygiene [12]. On the other hand, recent evidence associates periodontal disease with SCL [15-17]. Salivary levels of cortisol have been used as a marker of CS and depression in the field of psychology [22], as well as to assess the association between CS and periodontitis [11,13,15-17]. Some previous studies have shown a positive association between SCL and periodontitis [15-17]. Nevertheless, it is still not clear whether CS can also interact with CP in adult subjects.

To date, since there is still very little information assessing the association between CS and CP, further investigation can contribute to evaluating this hypothesis. To fill this research gap, the aim of this study was to assess the association between emotional stress, SCL, and CP.

MATERIALS AND METHODS

Study design and population

Consecutive systemically healthy (25 males and 45 females), nonsmoking patients, 30 to 65 years old, were assessed for inclusion in the study from January to September 2011. They were divided into CP patients and healthy controls. The subjects were selected among patients referred to the dental clinic for treatment, School of Dentistry, University of San Martín de Porres, Lima, Peru.

Exclusion criteria were individuals who chronically used corticosteroids and/or immune suppressor drugs as well as those with immunosuppressive diseases, individuals who used antibiotics within the last 6 months, those who had symptoms of acute illness, and individuals with fewer than 3 or fewer natural teeth. Subjects with known systemic conditions that could interfere with periodontal disease and who had undergone antibiotic or periodontal treatment in the previous six months were not included. Smokers were also excluded from the study.

The subjects participating in this cross-sectional study were volunteers who received detailed information about the proposed research and gave informed consent (i.e., signed a consent form to participate in the study). In addition, the study was approved by the Research Ethics Committee of the university and conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Clinical measurements

Upon admission, full medical and dental histories were obtained from the participants. All clinical measurements were recorded by one examiner (M.R.) using a University of North Carolina 15 style periodontal probe. The following measurements were recorded: 1) probing depth (PD); 2) clinical attachment level (CAL); 3) bleeding on probing (BOP); and 4) dental mobility. Based on a full mouth examination, the measurements of six sites around each tooth were recorded for all teeth and rounded to the nearest 0.5 mm. Readings were repeated by the same examiner (M.R.) in order to perform an intraobserver reproducibility analysis.

Periodontal diagnosis and experimental groups

The periodontal diagnosis of the participants was based on the 1999 American Academy of Periodontology (AAP) classification system (AAP 1999). Based on the periodontal measurements, the eligible subjects were divided into one of the following groups: 1) control subjects with no history of periodontitis, no sites with PD, and CAL>3 mm concomitantly (n=34); and 2) subjects with localized (<30% of teeth were affected) or generalized (>30% of teeth were affected) CP (n=36). CP was defined as >3 sites with a probing pocket depth of >4 mm and BOP, and the severity of disease was classified on the basis of the amount of attachment loss (i.e., slight, 1 to 2 mm; moderate, 3 to 4 mm; and severe, >5 mm) (AAP).

Therefore, 2 groups of patients were studied: the CP subjects versus the healthy controls.

SCL sampling and stress assessment

Saliva samples were collected from all subjects between 9 AM and 11 AM to minimize any circadian rhythm effects. The participants were asked not to eat, drink, or brush their teeth in the overnight period before collection. Collection of 1 mL unstimulated whole saliva was performed using sterile tubes. Patients with removable partial dentures kept them in their mouth during saliva collection. After that, patients were asked to complete the Self-rating Depression and Anxiety Scale about their stress level, depression, and anxiety [23,24].

Samples were stored at –20°C and salivary cortisol was assayed within the first month after collection. The quantitative determination of cortisol in saliva was assessed using a highly sensitive electrochemiluminescence immunoassay

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for salivary cortisol measurement (Roche Diagnostics, Mannheim, Germany) and a modular analytics analyzer (Elecsys Modular Analytics E170, Roche Diagnostics, Tokyo, Japan). Salivary cortisol levels were considered to be normal (1.64 to 6.00 ng/mL) or high (>6.00 ng/mL).

Statistical analysis

Descriptive statistics were used to synthesize the collected data. The means and standard deviations for the SCL were calculated for both groups using each patient as a unit of analysis. The odds ratio (OR) chi-squared test was applied to assess whether the SCL values were consistent between one group and another (that is, whether the levels of cortisol were similar between subjects with or without CP). Also, the 95% confidence limit was calculated, and the one way analysis of variance was used to evaluate differences between the SCL within subjects with CP according to the severity of disease. If a significant *P*-value was reached, the Tukey-Kramer method was used for pairwise comparisons to identify which means were significantly different from one another. Differences at $P<0.05$ were considered statistically significant.

In addition, the level of depression was quantified via Zung's Self-rating Depression Scale [23,24]. The scale comprises a 20-item questionnaire by which each participant rated his/her psychological condition related to physical conditions, emotional feelings, and somatic symptoms. On this scale, 10 negatively and 10 positively worded questions were presented and subjects were asked to provide one of the following responses: 1) "a little of the time"; 2) "some of the time"; 3) "a good part of the time"; or 4) "most of the time" [23,24]. It was observed that the outcomes of the instrument were in line with SCL outcomes.

RESULTS

A total of 36 subjects with CP (51.4%) and 34 without CP were evaluated (Table 1). Of them, all of the subjects with CP and one periodontally healthy subject were diagnosed with depression and anxiety (according to the Zung Scale) (Table 2).

Out of the 34 subjects with CP, 12 (33.3%) had a diagnosis of slight periodontitis, 21 (58.4%) had a diagnosis of moderate periodontitis, and 3 (8.3%) had a diagnosis of severe periodontitis. The degree of association between the severity of CP and the SCL investigated by statistical analysis showed that there was a significant difference between subgroups ($P=0.02$). Following the pairwise comparison of means (Table 3), it was identified that subjects with moderate CP had statistically significantly higher levels of salivary cortisol than subjects with a diagnosis of slight CP ($P=0.006$). Also, subjects with severe CP showed the same outcome when compared to those with slight CP ($P=0.012$).

In addition, Table 4 shows that 46 subjects (65.6%) presented high SCL whereas 24 had a normal level (34.3%). CP was found to be correlated with the SCL, with an OR of 4.14 (95% confidence interval, 1.43 to 12.01).

DISCUSSION

In this cross-sectional study, the association between depression, CP, and SCL was assessed. As reported in Table 4, a

Table 1. Distribution of subjects according to the presence of anxiety and chronic periodontitis.

Anxiety	Chronic periodontitis, n (%)		
	With	Without	Total
With	30 (42.85)	26 (37.15)	56 (70.00)
Without	6 (8.58)	8 (11.42)	14 (20.00)
Total	36 (51.43)	34 (48.57)	70 (100)

Table 2. Distribution of subjects according to the presence of depression and chronic periodontitis.

Depression	Chronic periodontitis, n (%)		
	With	Without	Total
With	36 (51.43)	1 (1.42)	37 (52.85)
Without	0 (0)	33 (47.15)	33 (47.15)
Total	36 (51.43)	34 (48.57)	70 (100)

Table 3. Salivary cortisol levels (ng/mL) according to the severity of disease.

Chronic periodontitis	No.	Mean	SD	*P*-value
Slight	12	6.31	2.23	0.02[a]
Moderate	21	11.71	5.40	
Severe	3	15.25	4.19	

SD: standard deviation.
Tukey test: slight versus moderate ($P=0.006$); slight versus severe ($P=0.012$); moderate versus severe ($P=0.421$).
[a]One way analysis of variance.

Table 4. Association between salivary cortisol levels (normal or high) and periodontal diagnosis (subjects with or without chronic periodontitis).

Level of cortisol	Chronic periodontitis, n (%)		
	With	Without	Total
High	29 (41.43)	17 (24.29)	46 (65.71)
Normal	7 (10.00)	17 (24.29)	24 (34.29)
Total	36 (51.43)	34 (48.57)	70 (100)

$\chi^2=7.24$; degrees of freedom=1; $P=0.007$; odds ratio, 4.14; 95% confidence interval, 1.43 to 12.01.

strongly significant association between SCL and the presence of CP was found. Such an outcome suggests that psychoneuroimmunologic factors may play a role in the development of periodontal disease, which is in line with previous data [10,12-17].

The association between periodontitis and cortisol is not yet well-established. Very few human studies have reported an association between SCL and periodontal disease. Genco et al. [10], using a study sample of patients with and without periodontal disease, were the first researchers to conduct such an investigation. These authors observed that the basal SCL was elevated in patients with periodontal disease; however, very few details on this relationship were given [10]. Another study by Hilgert et al. [13] showed a positive association between SCL and periodontitis. This investigation concluded that hypercortisolemia was associated with the presence of periodontal disease [13]. In a similar way, Goyal et al. [17] found that the SCL was associated with patients with periodontal disease, but its very important determine whether the patient is depressed or not or going through an episode of anxiety. Inversely, Mengel et al. [11] did not find a correlation among the levels of immunologic mediators (interleukin [IL]-1B, IL-6), cortisol, and stress; however, we found a relationship among these factors. Nevertheless, this author suggested that their results might have been related to the small group sample, as well as to the issue that the groups of patients with and without periodontitis were selected from a periodontal maintenance program [11].

With respect to the severity of periodontitis, its relationship with the SCL has also not been studied in depth. Hilgert et al. [13], Ishisaka et al. [15], and Ansai et al. [16] found a positive relationship between the SCL and CP, and also determined that hypercortisolemia (i.e., high amounts of circulating cortisol) was associated with the severity of periodontitis. In these studies, the SCL varied significantly in relation to the severity of periodontal disease [13,15,16].

It could be argued that the association between cortisol and CP may be linked to the inhibitory effect of the HPA axis on the inflammatory response because all of the components of the immune response are inhibited by cortisol.

During the activation of the HPA axis, the T-helper phenotype of a subject is influenced by the inhibition of IL-12 and the stimulation of IL-10 by the macrophages [21,25,26]. As a result, the periodontal tissues may be more vulnerable to periodontal pathogens in sites with periodontal inflammation, and thus such a condition could lead to the localized destruction of periodontal tissues [15,16,21]. On the other hand, some inherent conditions related to this study should be considered, such as the sample size (only 70 subjects were evaluated), the uncertainty related to the use of biomarkers as possible indicators of the presence of periodontal disease, and the study design (i.e., cross-sectional). Furthermore, it should also be taken into consideration that subjects under stress seem to have poorer oral hygiene levels, associated with a lack of periodic dental therapy [11-13]. Although subjects exhibiting severe periodontitis had presented a higher SCL and a higher degree of financial and emotional stress when compared to subjects with incipient or without periodontitis, they also demonstrated deficient oral hygiene [10,12].

In our study, it is very important to emphasize that we found a relationship among emotional depression, level of anxiety, cortisol saliva level, and the risk of having CP (OR, 4). Therefore, every patient that has this condition requires more care, and perhaps more continuous supportive therapy to reduce the risk of CP.

In conclusion, the present study showed a strong relationship between emotional depression, level of anxiety, and SCL on the one hand, and CP on the other; however, the mechanisms leading to such interaction effects are still not clear. In summary, within the limits of this study, this cross-sectional study comprising a group of subjects visiting a university-based practice provided further information on the association of stress and CP. These findings are consistent with previous studies, which have evaluated SCL among subjects with and without CP. Subjects with high SCL may be at an increased risk for CP. However, the relationship among stress, oral hygiene, and markers of periodontal disease remains somewhat unclear.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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