

EVALUATION OF LEPTIN LEVELS IN THE SERUM AND GINGIVAL CEVICULAR FLUID OF CHRONIC PERIODONTITIS PATIENTS

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ABSTRACT

Leptin is pro-inflammatory mediator associated with the pathogenesis of periodontitis. The objective of the current study was to assess the levels of leptin in serum and gingival crevicular fluid (GCF) in chronic periodontitis (CP) patients. This cross-sectional study was conducted in Dental Teaching Hospital, Umm Al-Qura University, Makkah, Saudi Arabia. Forty-five individuals participated in this study: 25 with chronic periodontitis patients and 20 periodontally healthy controls. The patients were selected based on the criteria of American Academy of Periodontology using probing depths, bleeding on probing, clinical attachment loss and radiographs. GCF and serum samples were collected to estimate the leptin concentrations using enzyme-linked immunosorbent assay kits. Serum leptin levels were markedly higher in chronic periodontitis patients than in the healthy group ($P < 0.00001$). Although GCF leptin levels of chronic periodontitis patients were higher than the healthy group but this difference was not statistically significant (P Value = 0.5198). In conclusion, these results indicate positive association between serum leptin concentrations and chronic periodontitis. However, there was no significant correlation between GCF leptin level and periodontitis. Further studies are required to confirm these finding.

Keywords: LEPTIN; chronic periodontitis; gingival crevicular fluid; serum

INTRODUCTION

Periodontal diseases are chronic inflammatory condition induced by dental biofilm accumulation on tooth surfaces (Genco and Borgnakke 2013). They are among the most prevalent microbial diseases and represent a major oral health problem worldwide (Petersen and Ogawa 2012).

Inflammation in periodontal diseases occurs due to interaction of genetic, environmental, host and bacterial factors. Interaction between bacteria in dental plaque and host immune responses leads to periodontal inflammation dominated by macrophages, B and T lymphocytes (Gemmell et al. 2001). Cytokines such as IL-1, IL-6, IL-17 and TNF- α in

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inflamed periodontal tissues are responsible for the loss of connective tissue attachment and bone loss (Fujihashi et al. 1993, Duarte et al. 2010, Zhao et al. 2011).

Recent evidence has correlated the periodontal diseases and systemic conditions such as cardiovascular diseases, diabetes and obesity (Seymour et al. 2007). Leptin is hormone/cytokine secreted into blood mainly by adipocytes. Leptin not only regulates body weight, but also has a role in immune homeostasis. Leptin can regulate the innate and adaptive immune responses. Leptin play a role in homeostasis of thymus, T helper 1 (Th1) development and production of acute-phase reactants (La Cava and Matarese 2004). It has been demonstrated that increased leptin secretion is associated with production of proinflammatory cytokines and some immune-mediated disorders such as autoimmune diseases (Fernández-Riejos et al. 2010). In addition, there were a significant correlation between serum leptin and IL-6 and C-reactive protein levels and non-surgical periodontal treatment decreased these cytokines (Shimada et al. 2010).

Recent studies correlated leptin and periodontal diseases. Several studies have reported that serum leptin concentration of chronic periodontitis patients was significantly higher than of the periodontal healthy subjects (Shi et al 2015, Liu et al 2013, Karthikeyan and Pradeep 2007, Gundala et al. 2014, Purwar et al. 2015). On the other hand, the level of GCF leptin is reduced in chronic periodontitis patients compared with periodontal healthy control (Johnson and Serio, 2001, Karthikeyan and Pradeep 2007, Liu et al. 2014) and in saliva (Purwar et al. 2015, Khorsand et al. 2016).

Hence, the aim of this study is to evaluate the concentration of human leptin in the serum and GCF in periodontal health and disease.

MATERIALS AND METHODS

This cross-sectional study was approved by the Institutional Review Board at Faculty of Dentistry,

Umm Al-Qura University. All patients were referred from screening clinic in Dental Teaching Hospital, Umm Al-Qura University to receive Comprehensive Dental Care. Written informed consent was obtained from each subject. We evaluated all patients, 25 with generalized chronic periodontitis and 20 patients with clinically healthy periodontium.

Chronic periodontitis patients were diagnosed according to the criteria of American Academy of Periodontology using probing depths, bleeding on probing, clinical attachment loss and radiographs as shown in table 1. Subjects who receive any periodontal treatment within the last six months were excluded.

Blood samples were drawn from each patient before periodontal examination. Gingival crevicular fluid (GCF) was collected before measurement of clinical periodontal parameters.

GCF sampling: 3 μ l of GCF was collected using micropipette capillary tubes. Then, GCF was diluted with 300 μ l of phosphate buffered solution in Eppendorf tube and stored under at -80 °C until laboratory analysis.

Serum samples: Blood was collected from all subjects in 3 ml tubes without anticoagulant. Blood samples were left undisturbed at room temperature for 30 min then centrifuged for 3000 rpm for 5 min to separate the serum. The serum was stored at -80 °C until laboratory analysis.

Human leptin enzyme-linked immunosorbent assays (ELISA): ELISA kit was purchased from Abcam, UK. Leptin was measured by ELISA according to manufacturer instructions.

Statistical analysis: Statistical analysis was done by using Graphpad prism. The quantitative data were presented in the following mean and standard deviation. Unpaired t test was used to test quantitative data. Significance was considered when P value \leq 0.05.

TABLE (1) Clinical periodontal parameters

Clinical periodontal parameters	Chronic periodontitis	Healthy
Plaque index	1.33 ± 0.34	0.68 ± 0.38
Probing depth [mm]	2.11 ± 0.65	0.79 ± 0.45
Clinical attachment loss [mm]	2.25 ± 0.79	0.0
Bleeding on probing	23.89 ± 18.51	4.89 ± 1.81

RESULTS

The levels of leptin were measured by ELISA. The mean values of serum and gingival cevicular fluid leptin in healthy subjects and chronic periodontitis

patients are shown in table 2. Generally, the levels of leptin in the serum were much higher than in GCF (P value < 0.00001).

As can be seen in figure 1, the mean serum leptin level was greatest for chronic periodontitis patients (3665±187.1 pg/ml) compared with that of the healthy control (454.4±10.84 pg/ml). The difference between the serum leptin levels of chronic periodontitis patients and healthy subjects was statistically significant (P value < 0.00001).

On the other hand, in figure 2, the mean GCF leptin levels of chronic periodontitis patients (11.18 ± 1.43 pg/ml) were higher than the healthy subjects (9.73±1.76 pg/ml) but this difference was not statistically significantly (P Value = 0.5198).

TABLE (2) Leptin levels in the serum and gingival cevicular fluid (GCF) in healthy subjects and chronic periodontitis patients.

	GCF Leptin		Serum Leptin	
	Healthy	Periodontitis	Healthy	Periodontitis
Mean ± SEM	9.73 ± 1.76	11.18 ± 1.43	454.4 ± 10.84	3665 ± 187.1
P value (Unpaired t test)	Periodontitis vs healthy is 0.5198		Periodontitis vs healthy is <0.0001	
P value (Paired t test)	Serum vs GCF leptin in healthy and periodontitis is <0.0001			

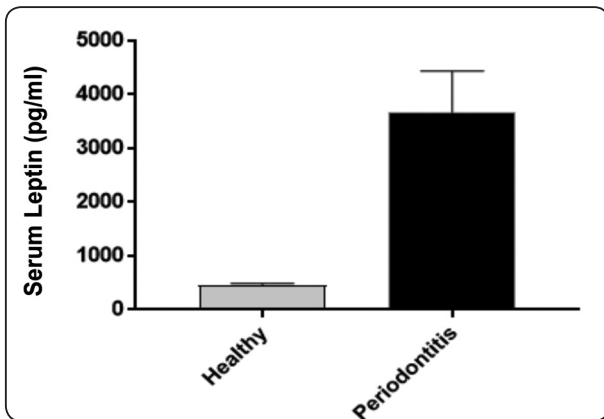


Fig. (1) Comparison of mean values of serum levels of Leptin in healthy subjects and chronic periodontitis patients. Serum leptin levels was measured by ELISA and Unpaired t test was used to compare the two groups (P < 0.00001).

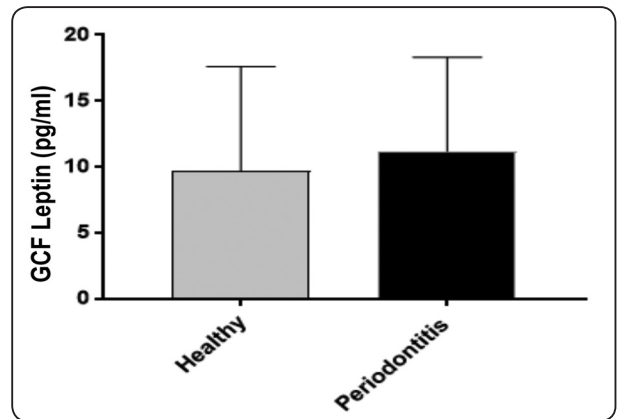


Fig. (2) Comparison of mean values of gingival cevicular fluid levels of Leptin in healthy subjects and chronic periodontitis patients. GCF leptin levels was measured by ELISA and Unpaired t test was used to compare the two groups (P value = 0.5198).

DISCUSSION

Leptin is pro-inflammatory mediator and may play a significant role in inflammatory responses. Leptin exerts its immunomodulatory effects through increasing secretion of pro-inflammatory mediators, and induction of Th1-cytokines production (La Cava and Matarese 2004, Procaccini et al. 2012). Lipopolysaccharide of Gram negative bacteria and inflammatory cytokines (IL-1, IL-6 and TNF- α) stimulate the adipocytes to produce leptin (Karthikeyan and Pradeep 2007a).

The current study evaluated the serum and GCF levels of leptin in healthy subjects and chronic periodontitis patients. We have shown that the leptin levels are much greater in the serum than in GCF. Although GCF leptin levels of chronic periodontitis patients were higher than the healthy group, but this difference was not statistically significant. The low levels of GCF leptin could be attributed to the absence of the adipocytes within gingiva despite high concentration of inflammatory cytokine in GCF during periodontitis (Karthikeyan and Pradeep 2007). Our GCF leptin results appear to contradict other studies that found an inverse correlation between the GCF leptin levels and chronic periodontitis (Karthikeyan and Pradeep 2007, Kanoriya et al. 2017). They suggest that the cytopathic changes in endothelial cells during inflammatory processes may cause decrease in GCF leptin.

The results of the present study shown that chronic periodontitis patients have a significant higher serum leptin levels compared with healthy group. These findings are in accordance with previous studies that found a positive correlation between serum leptin levels and periodontitis (Shi et al 2015, Liu et al 2013, Karthikeyan and Pradeep 2007, Gundala et al. 2014, Zhu et al. 2017).

The mechanisms through which periodontitis is correlated with serum leptin level remain ambiguous. However, the rise in serum leptin

concentration during periodontitis could be explained by three mechanisms. First, the source of serum leptin in periodontitis patients could be the gingiva and adipocytes and leptin might be released from gingiva concurrent to expansion of vascular network (Johnson and Serio, 2001). Secondly, the increases in leptin levels could be a host defense mechanism in response to periodontal pathogens (Arnalich et al. 1999).

Thirdly, TNF-alpha which is increased in periodontitis can stimulate leptin production through activation of the p55 TNF receptor (Finck and Johnson). leptin levels are higher in the serum than in GCF

CONCLUSIONS

In summary, our findings indicate positive association between the serum leptin concentrations and chronic periodontitis and serum leptin may be used as a risk marker for this disease. However, there was no significant correlation between GCF leptin level and periodontitis. Further studies are required to confirm these finding.

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Conflict of Interest Declaration

We have no conflict of interest to declare.

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