Original Article

Evaluation of Salivary Leptin Levels in Healthy Subjects and Patients with Advanced Periodontitis

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Abstract

Objectives: Leptin is a hormone-like protein produced by the adipose tissue. It plays an important role in protection of host against inflammation and infection. Some studies have reported changes in leptin levels in the gingival crevicular fluid (GCF), saliva and blood serum of patients with periodontal disease compared to healthy individuals. The aim of the present study was to compare the salivary leptin levels in patients with advanced periodontitis and healthy individuals.

Materials and Methods: In this case-control study, the salivary samples of healthy individuals and patients with advanced periodontitis with clinical attachment loss >5mm were obtained using a standardized method and the leptin levels were measured in the salivary samples by means of ELISA. The effects of the periodontal status and sex on the salivary leptin levels of both groups were statistically analyzed by two-way ANOVA.

Results: The means ± standard deviation (SD) of salivary leptin levels in healthy subjects and patients with advanced periodontitis were 34.27±6.88 and 17.87±5.89 pg/mL, respectively. Statistical analysis showed that the effect of sex on the salivary leptin levels was not significant (P=0.91), while the effect of advanced periodontitis on the salivary leptin levels was significant compared to healthy individuals (P<0.0001).

Conclusions: In patients with advanced periodontitis, the salivary leptin levels were significantly lower compared to healthy individuals. Thus, assessment of salivary leptin can be done as a non-invasive and simple method to determine the susceptibility of patients to advanced periodontitis.

Keywords: Leptin; Saliva; Periodontitis; Enzyme-Linked Immunosorbent Assay

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INTRODUCTION

Periodontal disease is a major oral health-related problem that affects a large number of patients all over the world [1,2]. In this context, advanced periodontitis, with clinical attachment loss (CAL) of 5mm or more, affects approximately 10-15% of the adult population worldwide [3]. Based on some observations, the most important risk factors for periodontal disease include poor oral hygiene, tobacco use, excessive alcohol consumption, stress and diabetes [4].

Tissue-destroying active cytokines have been reported to be responsible for the loss of connective tissue attachment and bone loss. Different kinds of cytokines are synthesized in inflamed periodontal tissues by lymphocytes, monocytes and non-immune cells such as fibroblasts and epithelial and endothelial cells [4]. Cytokines are soluble glycoproteins that function as local signaling molecules to control and coordinate cell behavior and function.

A hormone-like protein, leptin, which initially attracted attention due to its important roles in regulating weight, metabolism and function of reproductive organs, might play a role in some inflammatory conditions through its direct effect

on innate and adaptive immune cells [5]. Leptin is an adipose-derived hormone secreted in proportion to the size and number of adipocytes. Evidence shows that plasma leptin levels increase in obese subjects and decrease after weight loss [6]. Although leptin is predominantly synthesized by the adipose tissue, other tissues also produce this adipokine at low levels. Leptin inhibits appetite, stimulates energy expenditure, and it can modulate lipid and bone metabolism, coagulation, hematopoiesis, function of pancreatic beta cells, insulin sensitivity, etc. [7,8]. Moreover, leptin regulates the immune system and inflammatory response in various organs [7-11].

Leptin is predominantly expressed in the adipose tissue and is considered an important signaling molecule for the regulation of food intake and consumption of energy. In addition, leptin is produced in placenta, granulocytes, stomach and salivary glands. Leptin is a 16D non-glycosylated peptide hormone classified as a cytokine, with structural similarities to cytokine families with long and intertwined chains, such as interleukin-6 [12]. Therefore, it has been suggested that leptin might modulate the host response to infectious agents and play a role in mounting inflammatory responses in the body through induction of synthesis of other proinflammatory cytokines and stimulation of phagocytosis by macrophages [13]. The biologic functions of leptin include its antilipemic effects, its effects on reproduction, hematopoiesis, angiogenesis, blood pressure, osteogenesis and homeostasis of the lymphoid organs and T lymphocyte system. Johnson and Serio [14] in 2001 showed the presence of leptin in healthy gingival tissues and in the gingiva with marginal inflammation, which decreased with the progression of inflammation and an increase in pocket depth. In their study, apart from the adipose cells, the gingiva was also reported to be a source of synthesis of leptin [14]. Karthikeyan and Pradeep [15] in 2006 reported a significant decrease in leptin levels in the GCF with an increase in the destruction of periodontal tissues. In addition, the same researchers in another study showed that contrary to the GCF, the serum leptin levels had a direct relationship with chronic periodontitis [16]. In another study by Sattari et al, [17] in 2011 leptin was not reported to have an inflammatory role in periodontal diseases. Overall, it appears that the expression of leptin gene can have an important role in the modulation of inflammatory processes during infection, similar to the response of other cytokines to tissue injuries and infection [18]. The present study was carried out to compare the salivary leptin levels in healthy individuals and patients with advanced periodontitis.

MATERIALS AND METHODS

A total of 16 advanced periodontitis patients with a mean age of 32.06±3.70 years (range 26-37 years) were selected among the patients referred to the Department of Periodontology at Tehran University of Medical Sciences (TUMS), School of Dentistry. The study was conducted from March 2013 to June 2014. Healthy control group consisted of 16 individuals with a mean age of 32.00±3.44 years (range 28-39 years). Periodontal parameters examined in this study were plaque index (PI), probing pocket depth (PPD), CAL and bleeding on probing (BOP). The subjects underwent clinical examination by a periodontist using a Williams probe (Hu-Friedy, Chicago, IL, USA). Patients with advanced periodontitis, intraoral radiographic evidence of bone loss and CAL more than 5mm (according to the American Academy of Periodontology International Workshop for Classification of Periodontal Diseases, 1999) and healthy individuals were evaluated. The exclusion criteria were systemic diseases, recurrent infections, allergic reactions, cancer, use of medications affecting periodontal status during the previous three months, use of antibiotics during the previous two months, history of acute psychological stress

Fig. 1: Whole saliva collection

during the previous year, history of transplantation, drug abuse, history of radiotherapy, smoking, pregnancy, breastfeeding, menopause, acute malnutrition and history of periodontal therapy in the previous six months. Then, the study protocol was explained to the subjects and written informed consent was obtained from those who agreed to participate in the study. The saliva samples were collected based on the California University protocol [19] (Fig. 1).

The patients were asked to abstain from eating and drinking (except for water) for one hour before collecting unstimulated whole saliva samples. To obtain more accurate results, the subjects were asked to abstain from smoking, chewing gums and any activity that stimulates saliva secretion for one hour before collection of samples. Before collecting the samples, the patients rinsed their mouth with drinking water and evacuated their mouth without excessive movements of their head and remained seated for five minutes. Then, after a deep swallowing action, they evacuated the saliva from their mouth by bending positioned to collect saliva samples, leaving their mouth slightly open so that saliva slowly flowed into the test tube. The saliva samples were stored in a freezer at -40°C until all samples were collected [19]. The salivary leptin levels were determined based on the instructions providedin the kit (R&D Systems, Minneapolis, MN, USA).

Statistical analysis

The statistical analyses were carried out with two-way ANOVA. Spearman's correlation coefficient was used to evaluate the correlation between the salivary leptin level and various clinical parameters. Type I error was set at $P<0.05$.

RESULTS

The age, sex and BMI of patients and healthy controls are depicted in Table 1. There was no significant difference ($P>0.05$) in the mean age between the study groups. Regarding the mean BMI, the current results found no significant difference ($P>0.05$) in the mean BMI between the study groups; the mean BMI was 22.29±1.42 kg/m^2 in the periodontitis group, and this value was 21.94±1.36 kg/m^2 in the healthy control group. The differences in clinical periodontal parameters in patients and healthy controls are summarized in Table 2. The mean values of PI, PPD, CAL and BOP were significantly higher ($P<0.001$) in the periodontitis group compared to the healthy control group. The mean ± SD salivary levels of leptin in the healthy individuals and patients with advanced periodontitis were 33.58±6.64 (range 21.0-42.5) and 18.08±6.01 pg/mL (range of 8.0-27.0), respectively. In addition, the mean ± SD salivary levels of leptin in the healthy females and males with advanced periodontitis were 18.8±5.87 pg/mL (range 26.0-12.5) and 17.72±6.26 pg/mL (range 8.0-27.0), respectively (Table 3). The results of two-way ANOVA showed that the effect of sex on salivary levels of leptin was not significant ($P=0.91$); however, the effect of advanced periodontitis on salivary levels of leptin was significant compared to healthy individuals ($P<0.0001$). The interaction effect of sex and advanced periodontitis on salivary leptin levels

Table 1: Age, sex and BMI of subjects in the study groups

		Healthy control	Periodontitis	P-value
Age (years)	**Range**	28-39	26-37	
	Mean ± SD	32.00±3.44	32.06±3.70	0.961*
Sex	**Male**	8	8	
	Female	8	8	
BMI (kg/m^2)	**Mean ± SD**	21.94±1.36	22.29±1.42	0.488*

*Not significantly different (P>0.05). SD: Standard deviation

was not significant (P=0.98). The correlation between leptin levels and clinical periodontal parameters is shown in Table 4.

DISCUSSION

Leptin is a hormone-like protein, which initially attracted attention due to its significant role in regulating weight and metabolism of the human body [20]. However, subsequent research showed that leptin affects the body's defense mechanisms, including T cells, macrophages and endothelial cells. Therefore, some researchers have dubbed it a cytokine [20,21]. In addition, some studies have called it an acute-phase inflammatory protein [22,23]. On the other hand, the salivary glands of mammals secrete leptin and high levels of epidermal growth factor and other growth factors daily into the oral cavity. Since cells in the area, which have been traumatized (or affected by periodontal disease) can also synthesize growth factors with a role in the disease progress or wound healing, evaluation of mechanisms related to their protective effects on the course of periodontitis or other oral complications is of great importance [24].

Based on the available evidence, the salivary cytokines play a direct role in immune mechanisms in the oral cavity. For example, leptin, which is a member of IL-6 family prevents a decrease in the synthesis of mucin in salivary glands after the activation of lipopolysaccharides [25]. Therefore, salivary leptin plays a role in the effect of mucin on preventing the growth of bacteria. Such preventive effects of leptin on bacterial growth have been reported in the respiratory mucosa of mice [26], indicating the mucosal secretion of cytokines and their important role in preserving health.

The salivary hormones not only modulate the activity of immune cells, but also they play a role in the activity of the oral immune system against prokaryotes. Recently, use of saliva instead of blood for different diagnostic purposes has attracted attention [27,28] because the process of saliva sample collection is non-invasive and is preferred by many individuals [28]. In this context, in the present study the salivary leptin levels were determined in healthy subjects and patients with advanced periodontitis. Based on the results of the current study, there was a decrease in leptin salivary levels by 50% in periodontitis patients compared to healthy controls. The majority of previous studies have

Table 2: Clinical periodontal parameters

Clinical periodontal parameters	Healthy control*	Periodontitis*
Plaque index	0.56±0.63	2.44±0.63
Probing pocket depth (mm)	1.13±0.62	6.13±1.78
Clinical attachment loss (mm)	0	5.94±0.77
Bleeding on probing	5.94±5.23	41.88±18.52

***** In all parameters P<0.001

Table 3: Descriptive statistics of salivary leptin levels in healthy subjects and patients with advanced periodontitis

Group	Range	Median	Mean	SD
Healthy	21.00-42.50	33.70	33.58	6.64
Periodontitis	8.00-27.00	18.77	18.08	6.01
P-value			<0.01	

evaluated the serum levels of leptin in healthy individuals and during periodontal disease, using GCF. To the best of our knowledge, only a few studies have compared the salivary levels of leptin in healthy subjects and patients with periodontitis [29,30].

Purwar et al, [29] in 2015 evaluated leptin levels in saliva and serum of patients with chronic periodontitis and healthy controls. They concluded that the level of salivary leptin in chronic periodontitis patients was significantly lower than that in healthy controls, consistent with the results of the current study. They also noticed that the level of serum leptin increased in patients with periodontitis compared to healthy volunteers. In another study in 2015, Purwar et al, [30] found that appropriate non-surgical therapy can increase the leptin levels in the saliva of chronic periodontitis patients. Selvarajan et al, [31] in 2015 examined and compared the GCF concentration of leptin in patients with periodontal disease and healthy subjects. They observed a substantial decrease in leptin levels in GCF as the periodontal disease progressed. The authors suggested a potential protective role for leptin with regard to periodontal health. However, with the progression of periodontal disease, the protective effects of leptin on gingival tissues decrease due to a decrease in its concentration. Karthikeyon and Pradeep [16] in 2007 reported a significant decrease in the leptin concentrations of GCF with an increase in periodontal tissue destruction, with the highest and lowest leptin concentrations in the GCF in healthy subjects (2658 pg/mL) and patients with chronic periodontitis (1312 pg/mL), respectively. In their study, GCF samples were evaluated, which are derived from the gingival blood vessels; however, in the current study, salivary samples from healthy subjects and patients with advanced periodontitis were evaluated [16]. On the other hand, Johnson and Serio [14] in 2001 evaluated leptin concentrations in healthy gingival tissues with a gingival sulcus index of ≤3mm and in inflamed gingiva with periodontal pockets >3mm, with the use of ELISA technique. The results showed the highest leptin concentrations in the gingiva adjacent to the sulcus with a maximum depth of 3mm [14]. Based on the results, leptin was present in the healthy and inflamed human gingival tissues and its concentration decreased with an increase in pocket depth and aggravation of periodontal disease, consistent with the results of the present study, in which the salivary leptin levels were higher in healthy subjects compared to patients with advanced periodontitis. Bozkurt et al, [32] in 2006 evaluated leptin levels in the GCF and the effect of smoking on these levels. They reported lower leptin levels in GCF in smokers compared to non-smokers. It appears that smoking disrupts the mechanism regulating leptin levels. In the current study, smokers were excluded from the study. Therefore, the effect of smoking, as a confounding factor, on the results was eliminated. In another study, the concentration of leptin was determined in the GCF in healthy periodontium, in periodontium with chronic gingivitis and in chronic periodontitis. Based on the results, there was an inverse correlation between leptin concentration in the GCF and the progression of periodontal disease, with leptin levels decreasing in the GCF with the progression of periodontal disease [15].

Table 4: Correlation between leptin levels and clinical periodontal parameters

Parameters	R-value	P-value
Plaque index	-0.692	<0.001
Probing pocket depth	-0.789	<0.001
Clinical attachment loss	-0.739	<0.001
Bleeding on probing	-0.647	<0.001

Aydin et al, [33] in 2005 evaluated salivary and plasma leptin levels in healthy young subjects and reported lower salivary levels compared to plasma levels, with slightly lower salivary and plasma leptin levels in males compared to females.

Based on the results, there were no significant differences in salivary leptin levels between males and females in our study, consistent with the results of previous studies. Another study showed a decrease in GCF leptin levels at one and 24 hours after orthodontic tooth movement, with an increase after 168 hours, which was attributed to changes in the periodontium after orthodontic tooth movement. The worsening of gingival tissue status during the early stages of orthodontic treatment is manifested by a decrease in GCF leptin levels [34]. Gangadhor et al, [35] in 2011 reported a significant decrease in gingival leptin concentrations and a significant increase in plasma leptin levels concomitant with the progression of gingival disease. In their study, a decrease in gingival leptin levels in patients with periodontitis was attributed to its release into the blood serum. Since adipocytes are considered a source for the synthesis of leptin, the presence of leptin in the gingival tissue, as reported by the researchers, was an interesting finding because no evidence is available at present in relation to the synthesis of leptin by cells such as fibroblasts or epithelial cells in the gingiva. In addition, since it is not clear which cells produce leptin in the gingiva, its presence in the gingiva might be attributed to its entrapment in the gingival tissue subsequent to its release into the bloodstream. In their study, a significant decrease in salivary leptin levels in patients with advanced periodontitis was observed in comparison to healthy subjects, consistent with the current study; however, saliva samples were evaluated in the current study while samples were taken from gingival tissues in their study.

In the current study, samples were not taken from the plasma of healthy subjects and patients with severe periodontitis. However, lower salivary leptin levels have been reported compared to plasma levels [33,36,37]. Therefore, it is not possible to make a correct estimate of leptin plasma levels through determination of salivary leptin levels, which might be explained by the saturated leptin transfer mechanism in the salivary glands. Such a mechanism has also been reported in the blood-brain barrier [38]. Higher salivary leptin levels indicate its protective role in gingival tissues. However, the exact mechanism of its protective role has not been explained and this finding might be accidental, without any cause-and-effect relationship. In addition, the mechanism of its decrease in the saliva of periodontal patients has not been elucidated. It appears that leptin is used as a substrate during the inflammatory process; thus, its concentration decreases. In the current study, salivary samples were immediately evaluated after being catabolized by the enzyme using the ELISA technique; thus, it is possible that the leptin stored in the cells was released by the enzymatic effect and included in calculations in addition to free leptin. However, further studies are recommended to elucidate the mechanisms involved in changes in salivary leptin levels in periodontal diseases. In general, saliva contains a combination of materials secreted into the gingival sulcus, i.e. gingivo-crevicular transudate, saliva from the salivary glands, debris, bacteria, epithelial cells, hormones and mucus. The GCF is filtered by plasma and enters into the oral cavity by seepage from the very fine blood vessels in the gingival sulcus. It contains

components similar to immunoglobulins found in human plasma; however, their concentration is 800-1000 times less than those in the plasma. In addition, GCF contains high levels of IgG and IgM and small amounts of IgA, which have enriched glandular saliva to some extent [39].

This fluid has been used in many studies to estimate leptin concentrations in healthy gingival tissues and in gingival tissues affected by periodontitis. Salivary leptin exerts significant physiologic effects on oral keratinocytes. Such an effect has an important role in wound healing in the oral mucosa. The effect of saliva on wound healing has been shown in animals [40-42] and salivary cytokines such as leptin might have a role in the antimicrobial capacity of the saliva, in association with epidermal growth factor [43,44]. Leptin might affect some biological mechanisms as well, including immune and inflammatory reactions, hematopoiesis, angiogenesis, osteogenesis, and wound healing [45]. It also appears to have anti-inflammatory effects [46]. The protective effect of leptin on gingival tissues has been confirmed [16]. High concentrations of leptin in the GCF in healthy periodontal status, too, might have a protective role in this respect because leptin affects the immune system [47]. It promotes osteogenesis through its direct effect on osteoblasts [48]. Nokhbehsaim et al, [49] in 2014 evaluated the serum levels of leptin in patients with periodontitis with and without diabetic mellitus type II. They found a significantly positive correlation between serum leptin level and clinical periodontal parameters. The authors believe that the discrepancies observed between various studies could be attributed to the differences in the sample size of studies, differences in types of samples used for each study, differences in sample collection methods and the inflammatory state of patients.

CONCLUSION

In patients with advanced periodontitis, the salivary level of leptin decreases significantly. Thus, determination of salivary levels of leptin can be done as a simple and non-invasive technique to determine the susceptibility to advanced periodontitis.

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