J Periodont Res 2007; 42: 300–304

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2006.00948.x

Leptin levels in gingival crevicular fluid in periodontal health and disease

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Karthikeyan BV, Pradeep AR. Leptin levels in gingival crevicular fluid in periodontal health and disease. J Periodont Res 2007; 42: 300–304. © 2006 The Authors. Journal compilation © 2006 Blackwell Munksgaard

Background and Objective: A high concentration of leptin is associated with healthy gingival tissue, and the concentration of leptin decreases as periodontal disease progresses. However, to date, the leptin concentration in gingival crevicular fluid has not been documented. Hence, the present study was carried out to explore the presence of leptin in gingival crevicular fluid in periodontal health and disease, and to probe further into its possible role in periodontal disease progression.

Material and Methods: A total of 45 adult patients were selected, based on their body mass index, for the study. They were categorized into three groups of 15 patients each, based on their periodontal tissue status, as follows: group I (clinically healthy gingiva with no loss of attachment); group II (chronic gingivitis with no loss of attachment); and group III (chronic periodontitis). Gingival crevicular fluid samples of 1 μL were collected extracrevicularly using white color-coded 1–5 μL calibrated volumetric microcapillary pipettes from one site in each person, and samples were analyzed for leptin using a commercially available enzyme-linked immunosorbent assay kit.

Results: The concentration of leptin in gingival crevicular fluid of patients in group I (2292.69 pg/mL) was statistically higher ($p < 0.05$) than in those of groups II (1409.95 pg/mL) and III (1071.89 pg/mL). This suggests a negative correlation of gingival crevicular fluid leptin concentration with clinical attachment loss ($p < 0.05$).

Conclusion: As periodontal tissue destruction increased, there was a substantial decrease in gingival crevicular fluid leptin concentration. This observation extends our knowledge of the protective role of leptin in periodontal health.

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Key words: gingival crevicular fluid; immunity; leptin; periodontitis

Accepted for publication July 11, 2006

Periodontal diseases are multifactorial in etiology. Although microorganisms are implicated as the etiologic agent that induces inflammation, it is the chemical mediators of inflammation that play a pivotal role in the loss of connective tissue, as well as supporting alveolar bone (1). Cytokines such as interleukin-1β, tumor necrosis factor-α, prostaglandin E2, and, recently, leptin, have been shown to orchestrate the host response to infectious and inflammatory stimuli (2).

Leptin (*Ob*), a product of the *ob* gene, is a 16-kDa nonglycosylated peptide hormone. It is synthesized mainly in adipocytes (3), and in minor quantities by placenta (4), T cells (5), osteoblasts (6) and gastric epithelium (7), which regulate weight control and modulate other physiological functions, such as regulation of neuroendocrine, reproductive and haematopoietic systems, and bone remodeling.

Recently, leptin has been classified as a cytokine because it shows structural similarities to the long-chain

helical cytokine family, which includes interleukin-6, interleukin-11, and leukemia inhibitory factor (8). Moreover, leptin stimulates the immune system by enhancing pro-inflammatory cytokine production and phagocytosis by macrophages (9). Therefore, during infection and inflammation, leptin expression is modulated in a manner similar to the cytokine response to infection and injury. Thus, the overall increase in leptin during infection and inflammation indicates that leptin is part of the immune response and host defense mechanisms.

Alhough there are no adipocytes in gingiva, in a recent study by Johnson & Serio (10) it was shown that the leptin concentration is higher in the healthy gingiva compared with the diseased gingiva and they proposed that this might be caused by entrapment of leptin within the gingiva by diffusion from the microvasculature. As leptin has a role in the inflammatory response, an increased leptin level in healthy gingiva may be a host defense mechanism similar to that which occurs during sepsis (11). However, during gingival inflammation the concentration of leptin is decreased as a result of expansion of the vascular network caused by vascular endothelial growth factor, which may increase the net rate of leptin removal from the gingival tissues (10).

However, to date, the leptin concentration in gingival crevicular fluid in periodontal health and disease has not been explored. Hence, this study was designed to assess the concentration of human leptin in gingival crevicular fluid during periodontal health and disease, and, in addition, to obtain a more detailed insight into its possible role in the initiation and progression of periodontal disease.

Material and methods

Selection of patients

A total of 45 adult patients (20 men and 15 women), with a mean age of 37.2 years, who applied to the Department of Periodontology (Government Dental College and Hospital, Bangalore, India) for periodontal treatment, were admitted randomly to the study.

Based on the modified gingival index (12), Ramfjord periodontal disease index (13), and radiograph evidence of bone loss, patients were categorized into three groups. Group I (healthy) consisted of 15 patients who had clinically healthy gingiva with no loss of clinical attachment. Group II (chronic gingivitis with no loss of attachment) comprised 15 patients who showed clinical signs of gingival inflammation without any attachment loss. Group III (chronic periodontitis) comprised 15 patients who showed clinical signs of gingival inflammation with loss of attachment. Patients were recruited to the study if they had a normal body mass index, according to the chart of the World Health Organization 2002 (14), because obesity influences leptin levels in serum. Furthermore, the participants were free from medical complications, were not taking any medications affecting periodontal status, and had received no periodontal therapy in the preceding 6 mo. Exclusion criteria were: aggressive periodontitis; smoking; alcoholism; and/or pregnancy. Lactating women were also excluded from the study. The nature and procedures of the study were explained to, and informed consent was obtained from, all recruits. The Ethics Committee, Government Dental College, Rajiv Gandhi University of Health Sciences, approved the study protocol.

Gingival crevicular fluid sample collection

The test site selected, based on the periodontal status for sampling, was air dried, isolated with a roll of cotton, and supragingival plaque was removed without touching the marginal gingiva. Samples of gingival crevicular fluid were obtained before probing by placing white color-coded 1–5 μL calibrated volumetric microcapillary pipettes on the the site (Sigma Aldrich Chemical Co., Ltd, St Louis, MO, USA). From each test site, a standardized volume of 1 μL was collected, using the calibration on the micropipette, by placing the tip of the pipette extracrevicularly (unstimulated). Test sites from which no gingival crevicular fluid could be obtained, and the micropipette which was contaminated with blood and saliva, were excluded from the study. The gingival crevicular fluid collected was immediately transferred a plastic vial and frozen at −70°C until the time of assay.

Leptin assay

The assay was performed using the leptin Elisa kit (Biosource International Inc., Camarillo, CA, USA). The manufacturer's instructions were strictly adhered to and each plate was checked before use to ensure the calibration curve measured leptin standards (0–1000 pg/mL) within the stated limits of the assay. The kit made use of biotin conjugate and human leptin antibody. The samples were run in duplicate. Absorbance of the substrate color reaction was read on an enzyme-linked immunosorbent assay (ELISA) reader (Molecular Dynamics, Sunnyvale, CA, USA) using 405 nm as the primary wavelength. The optical density values obtained with the known samples were used to calculate the quantity of leptin in the other samples.

Statistical analysis

Descriptive statistics were used for each study group separately. The Kruskal–Wallis and Mann–Whitney *U*-tests were used to identify any difference in leptin levels between the groups.

Spearman's rank correlation tests were used to measure the association between leptin concentration, and the clinical parameters were analyzed using SPSS software, version 10 (SPSS Inc., Chicago, IL, USA).

Results

Leptin was detected in all gingival crevicular fluid samples. The healthy periodontium group demonstrated a significantly higher mean leptin concentration in gingival crevicular fluid (2292.69 pg/mL) compared with the chronic periodontitis group

(1071.89 pg/mL). The mean gingival crevicular fluid leptin concentration of the chronic gingivitis group (1409.95 pg/ml) was intermediate between those of the healthy and chronic periodontitis groups. The mean difference observed was statistically significant between the three groups, as shown in Table 1 and Fig. 1. When groups I and II, I and III, and II and III were compared, the differences were statistically significant (Table 2). From Table 3 and Fig. 2 it is evident that there was a significant positive correlation between leptin concentration and all clinical parameters tested (viz modified gingival index, periodontal disease index and clinical attachment loss). These results suggest that the leptin levels decreased progressively in gingival crevicular fluid from health to periodontitis. To correlate the body mass index with leptin levels, the participants in each study group were subdivided into three groups based on body mass index, as follows: 18.5 to < 20.5; 20.5 to < 21.5; and 21.5 to < 22.5. The mean body mass index observed among group I (20.6 ± 1.37), group II (20.7 ± 1.29), and group III (20.4 ± 1.15) was not significantly different ($p < 0.05$). The mean leptin levels were significantly different between the body mass index groups ($p < 0.05$) within each study group. Gingival crevicular fluid leptin levels also increased as the body mass index increased within each group, as shown in Fig. 3 and Table 4.

Table 1. Descriptive statistical values and Kruskal–Wallis test rank of leptin in the gingival crevicular fluid of the three study groups

Group	Mean (pg/mL)	SD	Minimum (pg/mL)	Maximum (pg/mL)	Kruskal–Wallis test (mean rank)	*p*-value
Group I	2292.69	187.29	1975.35	2596.13	38.0	< 0.0001*
Group II	1409.95	183.16	1073.64	1654.86	21.73	
Group III	1071.89	160.95	790.97	1356.74	9.27	

*The three means differ significantly at the 5% level of significance.

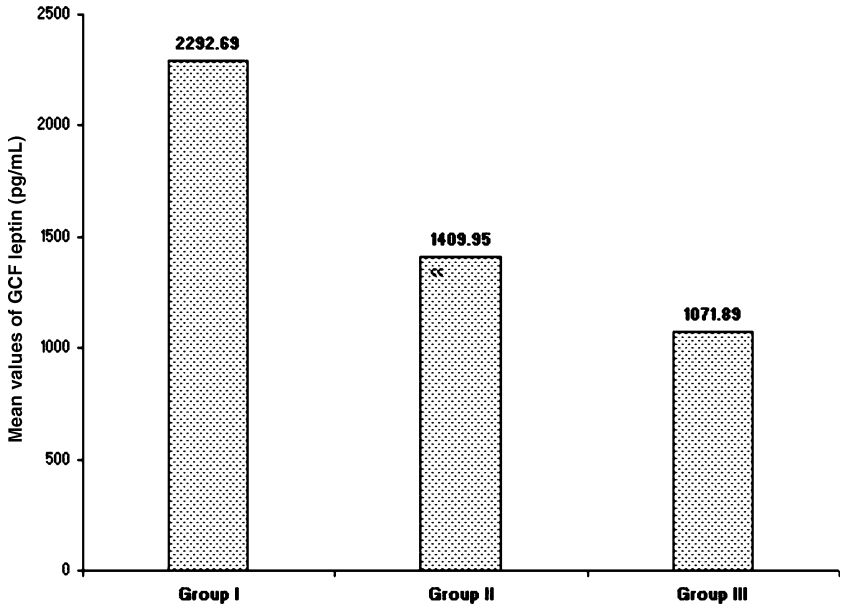

Fig. 1. Comparison of mean values of leptin in the gingival crevicular fluid (GCF) among the study groups. Group I, healthy gingiva with no loss of clinical attachment; group II, chronic gingivitis with no loss of attachment; group III, chronic periodontitis.

Table 2. Mann–Whitney U-test for Wise for comparison of mean leptin gingival crevicular fluid concentration

Group	Mean rank	*p*-value
Group I vs. Group II	23.0 and 8.0	
Group I vs. Group III	23.0 and 8.0	< 0.0001*
Group II vs. Group III	21.73 and 9.27	

*Significant at $p < 0.05$.

Table 3. Results of Spearman's Rank Correlation (*r*) coefficient

Pairs of variables	Leptin to MGI (*r*)	Leptin to PDI (*r*)	Leptin to CAL (*r*)
Group I	−0.96791	–	–
Group II	−0.93764	−0.90089	–
Group III	−0.96582	−0.77725*	−0.91678*

CAL, clinical attachment loss; MGI, modified gingival index; PDI, periodontal disease index.
*Significant at $p < 0.05$.

Discussion

Leptin is a hormone that is secreted into the blood in varying quantities by adipocytes and regulates weight (3). In addition, leptin enhances the body's immune mechanism by inducing the proliferation of human peripheral blood mononuclear cells (15), chemotaxis and oxidative species production by stimulated polymorphonuclear cells (15), phagocytosis by macrophages, secretion of interleukin-1R antagonist by human monocytes (*in vitro*, by 1.4-fold) (16), and the development/maintenance of natural killer cells (17). Furthermore, leptin is also involved in anti-osteogenic effects by acting centrally on the hypothalamus (18), but recently, leptin has also been suggested to play a role in bone formation by virtue of its direct effect on osteoblast proliferation, differentiation, and prolonging the life span of human primary osteoblasts by inhibiting apoptosis (19). Thus, leptin at a high concentration locally, protects the host from

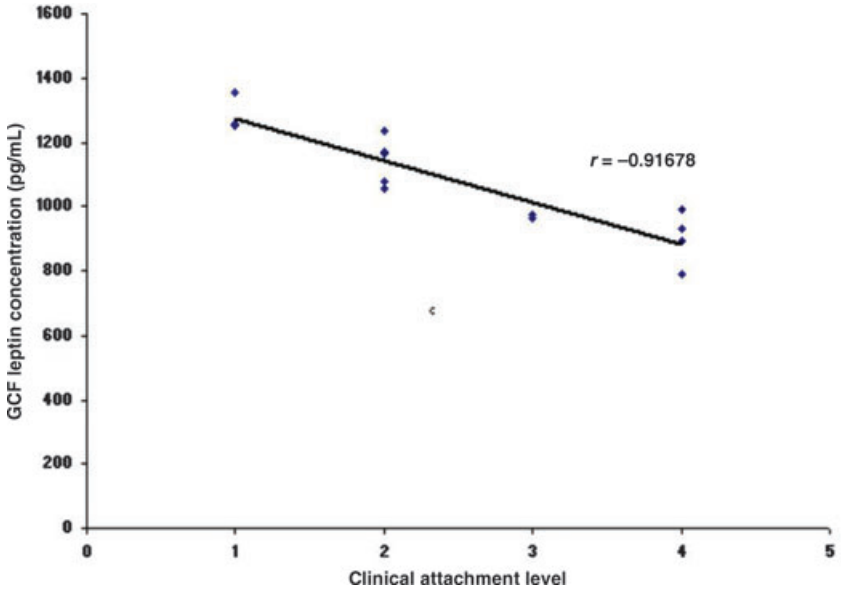

Fig. 2. Scatterplot of clinical attachment loss vs. leptin concentration in gingival crevicular fluid GCF) among the chronic periodontitis group.

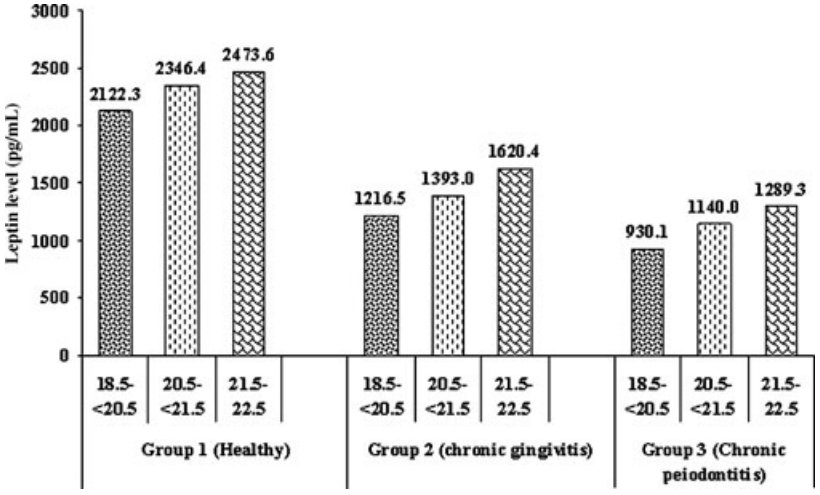

Fig. 3. Comparison of leptin level with respect to body mass index among the study groups.

inflammation and infection and maintains bone levels (7).

Recently, leptin concentrations in healthy and diseased gingiva were estimated and found to have a negative correlation between the leptin and interleukin-6 concentrations as periodontal disease progressed. This suggests that leptin is present within the healthy gingiva and that its concentration declines coincident with the severity of gingival inflammation and periodontal pocket formation (10).

The present study is the first to assess the concentration of human leptin levels in gingival crevicular fluid from healthy periodontium, chronic gingivitis, and chronic periodontitis patients. Furthermore, these concentrations were correlated to gain an insight into the possible role of leptin in the initiation and progression of periodontal disease. To avoid leptin derived from obese subjects biasing the estimation of leptin concentration, these subjects were excluded from the study by selecting only subjects with a normal body mass index (18.5–22.9 kg/m^2) according to a chart for the Asian population given by the World Health Organization in 2002 (14).

The results of the current study showed a mean gingival crevicular fluid leptin concentration in the healthy group of 2292.69 pg/mL, in the chronic gingivitis group of 1409.95 pg/mL, and in the chronic periodontitis group of 1071.89 pg/mL. Furthermore, from the statistical analysis it is evident that there is a strong negative correlation between the gingival crevicular fluid leptin concentration and periodontal disease progression. The results

Table 4. Descriptive statistical values and Kruskal–Wallis Test rank of leptin in the gingival crevicular fluid in relation to body mass index among the three study groups

Group	Mean (pg/mL)	Mean	SD	Minimum (pg/mL)	Maximum (pg/mL)	Kruskal–Wallis test (mean rank)	*p*-value
Group I	18.5 to < 20.5	2122.27	93.48	1975.35	2254.86	4.00	0.003
	20.5 to < 21.5	2346.35	0.70	2345.86	2346.85	8.50	
	21.5–22.5	2473.63	81.44	2385.76	2596.13	12.50	
Group II	18.5 to < 20.5	1216.45	98.21	1073.64	1324.65	3.00	0.002
	20.5 to < 21.5	1393.01	52.63	1342.75	1453.75	8.00	
	21.5–22.5	1620.38	49.82	1532.76	1654.86	13.00	
Group III	18.5 to < 20.5	930.08	69.04	790.97	990.75	4.00	0.002
	20.5 to < 21.5	1140.01	73.37	1056.12	1235.76	10.00	
	21.5–22.5	1289.25	58.45	1254.28	1356.74	14.00	

*Significant at $p < 0.05$.

obtained from our study are in accordance with the study carried out by Johnson & Serio (10), who also showed that leptin concentration is correlated negatively with the probing pocket depth.

Since leptin is produced by adipocytes, and gingiva contains no adipocytes, the presence of leptin within the gingival crevicular fluid is somewhat puzzling. The possible source of leptin in the gingival crevicular fluid may be serum (gingival crevicular fluid is considered to be a serum exudate) (20), gingiva (leptin entrapped within gingiva from the microvasculature) (10), and from the resident T cells and osteoblasts (a minor source) (6,7). The higher concentrations of gingival crevicular fluid leptin levels seen in periodontal health could be protective, as leptin stimulates the immune system (9) and enhances bone formation by acting directly on osteoblasts (7). Increased leptin levels are generally observed during inflammation, as a result of the stimulatory action of lipopolysaccharide (11) and cytokines, such as tumor necrosis factor-α (21) and interleukin-1α (22), on leptin production by acting on adipocytes. However, during periodontal inflammation there was no local rise in leptin concentration, probably as a result of the absence of adipocytes within gingiva to increase the leptin concentration when acted upon by these cytokines.

As the periodontal disease progresses, the protective effect of leptin on the gingiva is lost owing to a decrease in the leptin concentration (10). The mechanism underlying this decrease is not known; Johnson & Serio (10) linked leptin concentration negatively with inflammation. They speculated that, during gingival inflammation, the concentration of leptin is decreased as a result of expansion of the vascular network caused by vascular endothelial growth factor, which might increase the net rate of leptin removal from the gingival tissues and may raise the serum leptin levels. It may also be a result of leptin being used up as a substrate during inflammation, or it may be an artifact. Moreover, it has been suggested that an increase of the leptin concentration in serum acts as a risk factor for cardiovascular diseases because it promotes atherosclerosis (23). Based on this, it may be hypothesized that an increase in serum leptin concentration as a result of periodontal disease could act as one of the risk factors for cardiovascular disease. Further investigation has to be carried out to probe this aspect in greater detail (we are currently working on this project).

Within the limitations of our study, it can be postulated that the greater the extent of periodontal destruction, the lower the concentration of leptin in gingival crevicular fluid. This observation extends our knowledge of the protective role of leptin in periodontal health.

Acknowledgements

This research was partly supported by a research grant from Colgate Palmolive Research, India. The authors extend their gratitude to the subjects who participated in the study.

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