Acta Odontologica Scandinavica. 2015; 73: 633–641

informa
healthcare

ORIGINAL ARTICLE

The effects of periodontal therapy on serum and salivary leptin levels in chronic periodontitis patients with normal body mass index

PARTH PURWAR[1], MOHAMMED AKHLAQ KHAN[1], ABHISHEK GUPTA[2], ABBAS ALI MAHDI[3], SHIVANI PANDEY[3], BABITA SINGH[3], JAYA DIXIT[1] & PRIYA RAI[4]

[1]*Department of Periodontology, Faculty of Dental Sciences, King George's Medical University, Lucknow, Uttar Pradesh, India,* [2]*Department of Community Medicine, Hind Institute of Medical Sciences, Barabanki, Uttar Pradesh, India,* [3]*Department of Biochemistry, King George's Medical University, Lucknow, Uttar Pradesh, India, and* [4]*Private Practioner, Varanasi, Uttar Pradesh, India*

Abstract

Summary. Leptin concentrations are altered in favour of pro health after periodontal therapy. ***Background.*** Leptin, a non-glycosylated peptide hormone, not only maintains fat stores, but is also an integral part of host defense repertoire. Leptin levels have been found to be altered in an array of inflammatory diseases including chronic periodontitis (CP), but the role of non-surgical periodontal therapy (NSPT) in altering the leptin concentrations in saliva and serum of CP patients is yet to be ascertained. The aim of the present study is to quantify leptin levels in CP patients having normal body mass index (BMI) pre-therapy as compared to periodontally healthy controls and to address whether successful NSPT alters leptin concentration in serum and saliva. ***Materials and methods.*** Twenty-two saliva (modified draining method) and serum samples (by venipuncture) were collected from CP patients with normal BMI ($n = 22$), before and at 4 and 12 weeks after completion of NSPT, and periodontally healthy, age- and gender-matched controls ($n = 22$). Leptin levels were estimated using enzyme linked immunosorbent assay kits. ***Results.*** At baseline, CP patients had significantly different periodontal clinical parameters and the leptin concentrations in saliva of CP patients were found to be significantly lower than periodontally healthy volunteers (4710.10 ± 1133.21 vs 8721.10 ± 1019.58 pg/ml) ($p < 0.05$), whereas in serum the leptin concentrations were significantly higher than healthy controls (10749 ± 2062.24 vs 8085.00 ± 2859.68 pg/ml). Significant improvement in periodontal parameters, serum and salivary leptin levels were observed in CP patients at 4 and 12 weeks post-therapy ($p < 0.01$). ***Conclusion.*** Altered concentrations of leptin in serum and saliva are observed in CP patients which can be restored in favor of health after periodontal therapy.

Key Words: *Cardiovascular disease, leptin, non-surgical periodontal therapy, periodontitis, saliva, serum*

Introduction

Chronic periodontitis (CP), a chronic infectious disease of microbial etiology, produces an exaggerated inflammatory response to the pathogenic oral flora and affecting the attachment of connective tissue and supporting bone around the teeth, leading to tooth mobility and subsequent tooth loss [1]. Although micro-organisms are implicated as the primary etiological agents that cause the altered inflammatory response, it is the chemical mediators of inflammation, viz. cytokines, eicosanoids and matrix metalloproteinase, which play a critical role in the loss of connective tissue and the supporting alveolar bone [2].

Leptin, a 16 kDa non-glycosylated peptide hormone, shows structural homology with the long chain helical cytokine family (TNF-α, IL-6, IL-11 and leukemia inhibitory factor) [3] and shows cytokine-like expression during inflammation [4], immunity [5] and infection [6]. Overwhelming evidence currently conceptualizes adipose tissue as an endocrine organ rather than a simple storage of fat [7]. Leptin is synthesized predominantly in adipocytes [8] and in minor quantities by placenta [9], gastric epithelium [10], T-cells [11], osteoblasts [12] and intra-lobular

Correspondence: Dr Parth Purwar, BDS MDS, Senior Resident, C/O Dr D. N Purwar, Ankur Diagnostic Center, Lakhimpur Road, Gola Gokaran Nath, Kheri, Uttar Pradesh - 262802, India. Tel: +918765285612. E-mail: parth_94151@yahoo.com

(Received 1 February 2015; accepted 9 March 2015)

ISSN 0001-6357 print/ISSN 1502-3850 online
DOI: 10.3109/00016357.2015.1030768

ducts of major salivary glands [13]. Leptin, the 'fat sensor', monitors weight and modulates bodily functions such as glucose and lipid metabolism, thermo-genesis, neuro-endocrine function, reproduction, immunity, bone remodeling and cardiovascular function [14].

Previous studies have concluded that the leptin levels in gingiva [15] and gingival crevicular fluid (GCF) [16] in CP patients are significantly lower as compared to the healthy controls. On the contrary, in serum the leptin concentration has been found to be higher in CP patients [16] and non-surgical periodontal therapy (NSPT) has been found to be effective in reducing the serum leptin levels significantly [17]. The serum leptin levels are found to be positively influenced in favor of pro-inflammation in obese CP patients and may be considered as one of the risk markers for acute myocardial infarction [18,19]. Furthermore, longitudinal and cross-sectional studies have also shown positive association between elevated serum leptin concentrations and cardiovascular risks including stroke, acute myocardial infarction [20], chronic heart failure [21] and coronary heart disease [22]. In a recent cross-sectional study by Purwar et al. [23], the leptin levels in saliva of CP patients was found to be lower and in serum higher as compared to the healthy controls. The results also reflected that salivary leptin concentrations are inversely while serum leptin levels are directly related to the parameters of periodontal destruction.

In analogy with these observations, the present longitudinal study was conducted with two-fold aim: (1) to measure the concentration of leptin in saliva and serum from patients with normal BMI with and without CP and (2) whether leptin concentrations in CP patients can be altered to pro-health levels after periodontal therapy. Participants with normal BMI were selected for the study to avoid over-estimation of leptin from obese participants, which may bias the results of the study.

Materials and methods

The study population consisted of 44 volunteers (35–60 years) with normal BMI according to the chart of WHO [24] attending the Outpatient section of the Department of Periodontology, King George's Medical University, Lucknow, UP, India. The study was conducted in agreement with the principles embodied in the Helsinki Declaration of 1975, as revised in 2008 [25], and was approved by the institutional review board at the Medical University. The study was conducted in collaboration with the Department of Biochemistry, King Gerorge's Medical University, Lucknow. The study spanned over a period of 12 months, i.e. from March 2013 to March 2014. Before initiating the study, each patient was informed about the purpose and design of the study. Informed written consent and a thorough medical and dental history were taken from all the participants.

Patients were selected and enrolled in the study from among 124 individuals who visited the Outpatient Department of Periodontology. A total of 44 participants completed the study duration involving recall visits at 4 weeks and 12 weeks after NSPT. Twenty-two patients with generalized chronic periodontitis constituted the CP group (10 males and 12 females) and 22 healthy individuals (14 males and eight females) constituted the CG group. Exclusion criteria were five-fold: (1) use of tobacco in any form, (2) alcoholism, (3) pregnancy, (4) presence of any gross pathology and (5) patients who have received any periodontal therapy in the past 12 months or any anti-microbial, anti-inflammatory and immune suppressive therapy in the past 6 months prior to the study. Patients with less than 20 teeth present in the oral cavity and those who did not give their consent to be enrolled in the study were also excluded.

Sociodemographic characteristics of the study population

The independent co-variates included bio social and metabolic variables, frequency of use of oral hygiene aid and number of teeth present in the oral cavity. Biosocial variables comprise of age, gender, education, income and socio-economic status (SES) as assessed by Modified Prasad classification [26]. Modified Prasad classification divides SES into five classes: Class 1 having per capita monthly income of Rupees (Rs) (1€ ≈ 77 Rs) 5156 (67.02€) and above, Class 2 with income of Rs 2578 (33.51€)–5155 (67.01€), Class 3 with income of Rs 1547 (20.11€)–2577 (33.50€), Class 4 with income of Rs 773 (10.04€)–1546 (20.10€) and Class 5 with income less than Rs 773 (10.04€). Metabolic variables included measurement of body weight and height for assessment of BMI. The information on biosocial variables was obtained by means of well-structured written questionnaire to be filled by the participants.

Clinical examination

All the clinical parameters were assessed after the sample collection (saliva and blood) to avoid the contamination of the samples at baseline and at recall visits. The readings were noted by a trained and calibered examiner (PP). For each patient, plaque index (PI) [27], gingival index (GI) [28], probing depth (PD) and clinical attachment loss (CA loss) were noted at six sites (mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual) on each tooth excluding the third molars and the presence of bleeding on probing (BOP+) was evaluated using a sterile periodontal probe (PCP-UNC

15 periodontal probe, Hu-Friedy, Chicago, IL). All the clinical parameters were recorded by one independent examiner (PP) at baseline and at recall visits, 4 weeks and 12 weeks, twice in each appointment over a gap of 30 min. The intra-examiner agreement was assessed by kappa coefficient (κ), which was found to be >0.95 for all the periodontal parameters recorded.

Based on the assessed periodontal clinical parameters and radiographic evidence of bone loss, as evident by the orthopantomograms (OPG), the volunteers were categorized into: CP Group, which consisted of 22 participants with chronic periodontitis and CG Group, which consisted of 22 periodontally healthy participants. The diagnosis of chronic periodontitis was established on the basis of clinical findings of gingival inflammation, CA loss in excess of 5 mm, PD ≥4 mm at 3–4 sites in more than four teeth in each quadrant and radiographic evidence of bone loss. On examination more than 30% of the sites examined were positive for the above criteria and patients were categorized as generalized chronic periodontitis [29]. CG participants had no evidence of attachment loss, absence of clinical signs of gingival inflammation, no evidence of bone loss and had good oral hygiene status. The participants with aggressive forms of periodontitis were excluded from the study, although indicated periodontal management was rendered to them.

Collection of clinical samples

All the clinical samples were collected the following morning after patients had fasted overnight. Participants were asked to avoid brushing and drinking anything in the morning except water. Before sample collection, it was made sure that the individuals adhered to the aforementioned protocol by questioning the participants verbally. These protocols were followed during the entire study period.

Collection of saliva

Whole unstimulated salivary samples (~2 ml) were collected by modified draining method [30]. Patients were asked to expectorate into disposable polypropylene tubes every 30 s over a period of 5 min. The desired volume (~2 ml) of saliva was pipetted out in an eppendorf tube. The participants who were unable to expectorate the required volume of saliva were excluded from the study. Saliva samples were centrifuged at 4000× rpm for 10 min to remove the cell debris and 0.5 ml of the supernatant was stored in 1.5 ml aliquots, having a tracking number at 80°C until analysis.

Collection of serum

Two milliliters (~2 ml) of blood was collected from the ante cubital fossa by venipuncture using 20 gauge needles with a 5 ml syringe. Blood sample was allowed to clot at room temperature and, after 1 h, serum was separated from blood by centrifuging at 3000× rpm for 5 min and 0.5 ml of the extracted serum was immediately transferred to 1.5 ml aliquots. Each aliquot was designated a tracking number and stored at 80°C until further analysis.

Periodontal intervention

Non-surgical periodontal treatment (NSPT) included institution of oral hygiene instructions followed by the scaling and root planing (SRP) procedure, which was performed with the help of ultrasonic scalers (Aceton Satelec Suprasson P5 Booster, Dental Scaler, MerignacCedex, France) and hand curettes (Hu-Friedy scalers and curretes, Chicago, IL, U.S.A) by an experienced clinician (PP) for all patients belonging to the CP group. Each visit was of 30 min duration and the intervention was completed within 2 weeks after the enrollment of the participants in the study. Patients were recalled twice: at 4 weeks and at 12 weeks after the completion of NSPT for recording of clinical parameters and re-sample the clinical samples. During recall visits comprehensive oral hygiene instructions were reinforced. The patients were instructed to use only mechanical methods, i.e. toothbrushes and interdental cleaning aids during the study period and mouthwashes and/or antimicrobials were not prescribed.

The study incorporated four visits—one at baseline for completing well structured questionnaires regarding biosocial variables and instructions for sample collection and a second visit for sample collection, measurement of the periodontal parameters, weight and height and initiation of NSPT. Third and fourth visits were scheduled at 4 weeks and 12 weeks after the completion of NSPT.

Leptin analysis

Highly sensitive ELISA kits (Avibionhuman leptin ELISA kit, Ani Biotech Oy, Tillitie-3, Vantaa, Finland) were used to detect leptin levels in saliva and serum as prescribed by the manufacturer. Each plate was checked before use to ensure that the calibration curve measured leptin standards (0–1000 pg/ml) within the stated limits of the assay. The kit used made use of biotin antibody, HRP-Streptavidin solution and TMB Substrate. Absorbance of the substrate color reaction was read on ELISA reader (Life Sciences, Bio-Rad laboratories, California, U.S.A). Each patient was used as the unit of analysis. Leptin was detectable in all the clinical samples. The total leptin was determined in picograms (pg) and calculation of the concentration in each sample was performed by dividing the amount of leptin by the volume of sample (pg/ml). The standard curves of ELISA analysis has been provided as a supplemental file.

Table I. Sociodemographic, clinical and relevant characteristics of the study population.

Characteristics	CP		CG		*p*-value
	n	%	*n*	%	
Age (years)					
35–45	8	38.10	6	28.60	> 0.05
46–55	9	34.60	9	34.60	
55–60	5	26.30	7	36.80	
Gender					
Male	10	27.80	14	38.90	> 0.05
Female	12	40.00	8	26.70	
Education					
High school and below	7	41.20	4	23.50	> 0.05
Secondary education	10	34.5	10	34.50	
Post-secondary education	5	25.00	8	40.00	
*Socio-economic status (SES)**					
Class- 1	3	30.00	3	30.00	> 0.05
Class- 2	7	31.80	8	36.40	
Class- 3	4	25.00	6	37.50	
Class- 4	5	55.60	2	22.20	
Class- 5	3	33.33	3	33.33	
Number of teeth					
21–24	10	31.25	10	31.25	> 0.05
25–28	12	35.29	12	35.29	
Frequency of tooth brushing					
Once daily	4	36.40	3	27.30	> 0.05
Twice daily	14	34.10	14	35.70	
More than twice daily	4	28.60	5	35.70	
BMI in kg/m^2 (Mean ± SD)	20.45 ± 1.23			20.82 ± 1.67	> 0.05

*Modified Prasad Classification of Socio-economic Status.
CP, Chronic Periodontitis patients; CG, Periodontally healthy participants.

Table II. Clinical characteristics of patients with CP ($n = 22$) and periodontally healthy patients with CG ($n = 22$).

Clinical parameters	Mean ± SD	95% CI	Median	Min, Max	*p*-value
PI					
CP	1.73 ± 0.10	1.69–1.78	1.74	1.50, 1.89	< 0.05*
CG	0.33 ± 0.27	0.21–0.45	0.37	0.04, 0.76	
GI					
CP	1.81 ± 0.21	1.72–1.90	1.87	1.45, 2.16	< 0.05*
CG	0.38 ± 0.53	0.14–0.62	0.08	0.03, 1.54	
PD (in mm)					
CP	3.29 ± 0.90	2.89–3.69	3.10	1.49, 5.50	< 0.05*
CG	0.97 ± 0.69	0.67–1.28	0.65	0.15, 2.43	
CA loss (in mm)					
CP	3.54 ± 1.36	2.93–4.14	3.20	2.09, 5.98	< 0.05*
CG	1.15 ± 0.29	1.02–1.28	1.08	0.86, 2.09	
BOP (%)					
CP	65.60 ± 3.09	64.23–66.98	65.05	60.54, 70.65	
CG	3.79 ± 0.29	3.66–3.92	3.89	2.98, 4.00	< 0.05*
Sites with PD 4–5 mm (n)					
CP	48.53 ± 1.80	47.73–49.33	49.24	43.50, 51.45	–
CG	0	0	0		
Sites with PD >6 mm (n)					
CP	12.44 ± 0.46	12.23–12.65	12.50	11.78, 13.12	–
CG	0	0	0		

*Statistically significant compared with CG ($p < 0.05$) (One-way ANOVA test).
BOP, Bleeding on probing; CAloss, Clinical Attachment loss; CG, Periodontally healthy participants; CP, Chronic Periodontitis patients; GI, Gingival index; PD, Probing depth; PI, Plaque index.

Statistical analysis

Data entry and analysis was done using the Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc, Chicago, IL). Descriptive statistics such as mean and standard deviation (SD) for continuous variables and frequency and percentage for categorical variables were determined. The data was found to be normally distributed. One-way ANOVA and Paired *T*-test were used to determine the statistical significance. The level of significance was set up at $p \leq 0.05$.

Results

The patients fulfilling the inclusion criteria, giving their consent for the study and complying with the recall visits and the instructions regarding the sample collection were included in the study. Three patients (one in the CG group and two in the CP group) failed to comply with the follow-up schedule and, hence, are not included in the study results. Assuming an α error of 0.05, >95% confidence interval (CI) and leptin levels in saliva as the parameter, the power of the study was >90%.

Volunteer characteristics

Table I reveals that there were no statistically significant differences ($p > 0.05$) between the CP and CG groups in terms of age, gender, education, SES, number of teeth present, frequency of tooth brushing and BMI. However, the CP group ($n = 22$) constituted more participants belonging to lower SES, i.e. Class 4 and below (36.36%), and with education level up to high school and below (31.81%) as compared to the CG group (22.72% and 18.18%), respectively.

Clinical parameters

Descriptive statistics of the periodontal parameters at baseline of the study population are shown in Table II.

Table III. Biochemical characteristics of patients with CP (n = 22) and periodontally healthy patients CG (n = 22).

Biochemical parameters	Mean ± SD	95% CI	Median	Min, Max	p-value
Salivary leptin (pg/ml)					
CP	4710.10 ± 1133.21	4207.65–5212.53	5183.50	2567, 6000	< 0.05*
CG	8721.10 ± 1019.58	8269.00–9173.10	8761.80	6739, 9967	
Serum leptin (pg/ml)					
CP	10749 ± 2062.24	9834.65–11663.34	10289.00	8500, 16890	< 0.05*
CG	8085.00 ± 2859.68	6817.10–9353.00	7973.00	1224.80, 13100	

*Statistically significant compared with CG (p <0.05) (One-way ANOVA test).

At baseline, there were significant differences in mean values of PI, GI, PD, CA Loss, % of sites with BOP(+) between CP patients and healthy controls ($p < 0.05$).

Salivary and serum leptin levels

All the samples in each group tested positive for the leptin assay. The mean leptin concentration in saliva and serum obtained from the CP and CG groups are presented in Table III. The mean salivary leptin levels were significantly higher in the CG group (8721.10 ± 1019.58 pg/ml) than in the CP group (4710.10 ± 1133.21 pg/ml), whereas the mean leptin levels in serum were significantly higher in the CP group (10749 ± 2062.24 pg/ml) than in the CG group (8085 ± 2859.68 pg/ml). The analysis of variance showed that the differences in leptin concentrations in saliva and serum between the groups were found to be statistically significant ($p < 0.05$).

Comparison of the periodontal parameters in CP patients before and after treatment

PI, GI, CA Loss, % of sites with BOP(+) and sites with PD >4 mm decreased significantly from baseline to 4 weeks and 12 weeks after NSPT and the differences in their means were statistically significant ($p < 0.05$), as depicted in Table IV; however, there was an insignificant reduction in PD between the two recall visits.

Comparison of the biochemical parameters in CP patients before and after treatment

The patients in the CP group showed a significant elevation in salivary leptin levels from baseline (4710.10 ± 1133.21 pg/ml) to first recall visit (8179.86 ± 1403.61 pg/ml) and at second visit at 12 weeks (9092.50 ± 1264.66 pg/ml) after NSPT ($p < 0.05$). Furthermore, there was a significant reduction ($p < 0.05$) in serum leptin levels from baseline (10749 ± 2062.24 pg/ml) to both the recall visits at 4 weeks and 12 weeks (8253.68 ± 719.93 pg/ml and 6059.36 ± 1259.73 pg/ml), respectively, as depicted in Table V.

Table IV. Comparison of periodontal parameters at baseline and after therapy in patients with CP (n = 22).

Clinical parameters	Mean ± SD	95% CI	Median	Min, Max
PI				
Baseline	1.73 ± 0.10[1,3]	1.69–1.78	1.74	1.50, 1.89
4 weeks	0.89 ± 0.12[1,2]	0.83–0.95	0.91	0.65, 1.11
12 weeks	0.59 ± 0.25[2,3]	0.47–0.70	0.57	0.11, 1.08
GI				
Baseline	1.81 ± 0.21[1,3]	1.72–1.90	1.87	1.45, 2.16
4 weeks	1.09 ± 0.21[1,2]	1.00–1.19	1.07	0.87, 1.06
12 weeks	0.76 ± 0.09[2,3]	0.72–0.80	0.79	0.48, 1.03
PD (in mm)				
Baseline	3.29 ± 0.90[1,3]	2.89–3.69	3.10	1.49, 5.50
4 weeks	2.07 ± 0.23[1]	1.96–2.17	1.98	1.94, 2.78
12 weeks	1.65 ± 1.03[3]	1.19–2.10	1.65	0.23, 3.45
CA loss (in mm)				
Baseline	3.54 ± 1.36[1,3]	2.93–4.14	3.20	2.09, 5.98
4 weeks	2.80 ± 0.23[1,2]	2.69–2.90	2.75	2.45, 3.55
12 weeks	2.40 ± 0.59[2,3]	2.14–2.66	2.19	1.39, 3.92
BOP (%)				
Baseline	65.60 ± 3.09[1,3]	64.23–66.98	65.05	60.54, 70.65
4 weeks	22.57 ± 0.94[1,2]	22.16–22.99	22.56	20.77, 24.87
12 weeks	16.07 ± 1.14[2,3]	15.56–16.58	16.26	12.87, 17.87
Sites with PD 4–5 mm (n)				
Baseline	48.53 ± 1.80[1,3]	47.73–49.33	49.24	43.50, 51.45
4 weeks	35.60 ± 0.69[1,2]	35.34–35.95	35.50	33.78, 36.89
12 weeks	25.40 ± 0.93[2,3]	24.99–25.82	24.88	24.56, 26.98
Sites with PD >6 mm (n)				
Baseline	12.4 ± 40.46[1,3]	12.23–12.65	12.50	11.78, 13.12
4 weeks	10.51 ± 0.36[1,2]	10.35–10.67	10.40	9.98, 11.34
12 weeks	7.25 ± 0.41[2,3]	7.07–7.43	7.20	6.23, 8.02

[1,2,3]Paired t-test, Significant at $p < 0.05$.
[1]Indicates that their exists a significant difference in values at baseline and at 4 weeks.
[2]Indicates that their exists a significant difference in values at 4 weeks and 12 weeks.
[3]Indicates that their exists a significant difference in values at baseline and 12 weeks.
BOP, Bleeding on probing; CAloss, Clinical Attachment loss; GI, Gingival index; PD, Probing depth; PI, Plaque index.

Table V. Comparison of biochemical parameters at baseline and after therapy in patients with CP ($n = 22$).

Biochemical parameters	Mean ± SD	95% CI	Median	Min, Max
Salivary leptin (pg/ml)				
Baseline	4710.10 ± 1133.21[1,3]	4207.65–5212.53	5183.50	2567, 6000
4 weeks	8179.86 ± 1403.61[1,2]	7557.53–8802.19	8553.00	5430, 9987
12 weeks	9092.50 ± 1264.66[2,3]	8531.77–9653.22	9404.500	6016, 10793
Serum leptin (pg/ml)				
Baseline	10749.00 ± 2062.24[1,3]	9834.65–11663.34	10289	8500, 16890
4 weeks	8253.68 ± 719.93[1,2]	7934.47–8572.88	7943.00	7400, 9500
12 weeks	6059.36 ± 1259.73[2,3]	5500.00–6617.89	6328.00	3023, 8274

[1,2,3]Paired *t*-test, Significant at $p < 0.05$.
[1]Indicates that their exists a significant difference in values at baseline and at 4 weeks.
[2]Indicates that their exists a significant difference in values at 4 weeks and 12 weeks.
[3]Indicates that their exists a significant difference in values at baseline and 12 weeks.

Discussion

The present study focuses on the altered leptin concentrations in saliva and serum among chronic periodontitis (CP) patients with normal BMI and to gauge the effectiveness of non-surgical periodontal therapy (NSPT) in amending the leptin concentration towards health along with the improvement in the periodontal parameters. The study design involved generalized chronic periodontitis patients ($n = 22$) and healthy volunteers ($n = 22$).

The key finding of the present longitudinal interventional study reflected significant reduction of periodontal inflammation in CP patients with non-surgical periodontal treatment as evident by improvement in the periodontal parameters and decrease in serum leptin levels ($p < 0.05$), which is in accordance to the study by Shimada et al. [17]. To the best of the authors' knowledge, this is the first reported investigation that also depicted significant changes in leptin concentration in saliva from CP patients with normal BMI after NSPT.

The study focuses on chronic periodontitis, a condition characterized by chronic inflammation of the periodontal tissues, which in the current scenario is almost entirely diagnosed on the basis of an array of clinical measurements and radiographic findings. However, measurement of CA loss and assessment of radiographic bone levels provide an insight into the past periodontal breakdown rather than revealing current disease activity [31]. However, oral fluid-based biomarkers have the potential to provide an insight much beyond the classical clinical and radiographic findings of the disease process. These biomarkers can be either derived from soft tissue inflammation, alveolar bone loss, bacterial products or antimicrobial proteins associated with the periodontal destruction [32].

Saliva is an easily accessible biological fluid containing an array of biomolecules either produced locally or derived from the vascular beds in the gingival tissues. Moreover, saliva allows easy and safe sampling and salivary biomarker analysis reveals current disease activity, thereby making it a potential and viable alternative [33]. Henceforth, the need arises to further expand the profile of the potential biomarkers in the saliva that tends to alter significantly along with the periodontal parameters, can detect the presence of active disease, predict future disease progression, can be used to evaluate the response to periodontal therapy in conjecture with the periodontal parameters and can also provide an insight into the systemic health, thereby improving the clinical management of CP patients. In the current study, orthopantamograms (OPG) were used as an adjunct to clinical parameters for the diagnosis of CP. OPG provides an informative overall picture of the distribution and severity of bone destruction in periodontal disease, which is one of the limitation of the study as a complete intra-oral series is required for periodontal diagnosis.

It is exigent to understand the SES of the patients in order to correlate its impact on periodontal health. The Modified Prasad's classification takes into account the per capita monthly income and is applicable to both the rural and urban populations [26].

Recently, increasing evidence suggests the immunomodulatory role of leptin as it orchestrates the immune-inflammatory response similar to that of a cytokine [7]. In the present study the mean serum leptin concentration at baseline was found to be higher in CP patients as compared to the healthy controls (10749 ± 2062 pg/ml vs 8085 ± 2859 pg/ml), commensurate with the inflammatory profile of CP patients [34,35] and the difference in the means was found to be statistically significant ($p < 0.05$). The findings of the study are in accordance to the other studies in which plasma/serum leptin concentration was found to be higher during a plethora of inflammatory diseases including chronic periodontitis [16,17,19] and cardiovascular diseases (CVD) [20–22].

The elevated serum leptin levels in CP patients with normal BMI could be attributed to the stimulatory action of lipopolysaccharides from periopathogens and increased levels of cytokines (TNF-α and IL-1) on adipocytes, thereby increasing the leptin production and release into the systemic circulation [36]. Second, it could be a body defense mechanism to counteract periodontal inflammation, as leptin is an integral part of the immune response and host defense mechanism [37]. Gingiva could also contribute to the increased circulating leptin due to expression of vascular endothelial growth factor (VEGF) causing removal of leptin from gingiva into the circulation [15]. The increased serum leptin levels in CP patients in the study may be a combinatorial effect of the aforementioned factors. An increase in serum leptin levels may activate the inflammatory signaling pathway, leading to the generation of adhesion molecules like vascular cell adhesion molecule (VCAM-1), E- selectin and intracellular cell adhesion molecule (ICAM-1) [38]. This in turn increases the adherence of monocytes, which accumulate within the intima, and its continued accumulation leads to formation of a fatty streak, thus promoting atherosclerosis [39], neovascularization of atherogenic plaque [40] and induction of oxidative stress in the arterial wall [41]. Further, it induces the calcification of arterial walls, thus losing the elasticity and distensibility, making them stiffer and increasing the heart exertion, thereby increasing the workload on the heart [42]. It has already been hypothesized that a rise in serum leptin concentration up to 10,000 pg/ml in CP patients with increased BMI could be designated as a risk marker for future CVD [19]. In the current study the CP patients had serum leptin levels greater than 10,000 pg/ml. Although the cardiovascular status of the CP patients enrolled in the study was not evaluated, it can be speculated that elevated serum leptin levels in CP patients may lead to future adverse cardiovascular outcomes.

Previous investigations of leptin in human saliva indicated that leptin might play a decisive role in mucosal and host defenses [43]. Although there are no adipocytes in gingiva, Johnson and Serio [15] reported that leptin was present in healthy as well as in diseased gingiva. The salivary leptin concentration in the present study at baseline was found to be significantly lower ($p < 0.05$) in CP patients as compared to healthy volunteers (4710 ± 1133 pg/ml vs 8721 ± 1019 pg/ml), thereby depicting the protective role of leptin in the saliva, but the precise nature of the protective mechanism of leptin could not be ascertained. The source of leptin in saliva can be the intra-lobular ducts of major salivary glands or leaching of GCF constituents in saliva. The significant decrease in salivary leptin levels in CP patients may be due to the increased expression of leptin receptors during periodontal inflammation due to the cytopathic changes leading to the increased binding of leptin to the receptors, thereby decreasing the concentration of leptin in the saliva of CP patients. Also, leptin can be used as a substrate during periodontal inflammation; however, the exact mechanism of how it is protective is not known; it can also be coincidental rather than causal. Additionally, 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 [44]. Furthermore, leptin enhances the body's immune mechanism by inducing the proliferation of human peripheral blood mononuclear cells, chemotaxis and oxidative species production by stimulated polymorphonuclear cells [45] and the development/maintenance of natural killer cells [46]. Thus, leptin, at a high concentration locally, protects the host from inflammation and infection and maintains bone levels.

Four weeks after periodontal therapy there was improvement in all the periodontal parameters along with a significant ($p < 0.05$) reduction in serum leptin levels and an increase in salivary leptin levels (8253.68 ± 719.93 pg/ml and 8179.86 ± 1403.61 pg/ml, respectively). Twelve weeks after intervention it is conceivable that the non-surgical periodontal treatment was able to further amend leptin concentration in serum and saliva towards health (6059.36 ± 1259.73 pg/ml and 9092.50 ± 1264.66 pg/ml, respectively). The changes in periodontal parameters and leptin concentrations were due to reduction in periodontal inflammation achieved through NSPT, thus offering cardio-protective benefits to CP patients.

Taking these data together, it can be suggested that periodontal inflammation may up-regulate serum leptin and down-regulate salivary leptin levels in patients with CP. NSPT was able to restore the leptin levels and periodontal parameters on a short-term as well as long-term basis.

Conclusions

The following major conclusions can be drawn from this study: (1) higher levels of serum leptin were detected in patients with CP; (2) in the CP group, concentrations of salivary leptin were lower as compared to healthy controls; and (3) non-surgical periodontal therapy was able to amend leptin levels in saliva and serum towards health. However, non-availability of cardiovascular status of CP patients limited the scope of the study. Additionally, strict adherence to the inclusion and exclusion criteria restricted the sample size of the study.

This is a preliminary study; for more definitive conclusions, larger sample size studies are warranted. Additionally, correlation of salivary and serum leptin with inflammatory cytokines in CP patients is required to probe deeper into the aspect.

Acknowledgment

The authors extend their regards to the volunteers who participated in the investigation. No external funding was available for this study.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Savage A, Eaton KA, Moles DR, Eaton KA, Moles DR, Needleman I. A systematic review of definitions of periodontitis and methods that have been used to identify this disease. J Clin Periodontol 2009;36:458–67.
[2] Genco RJ. Host responses in periodontal diseases: Current concepts. J Periodontol 1992;63:338–55.
[3] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425–32.
[4] Faggioni R, Fantuzzi G, Fuller J. IL-I beta mediates leptin induction during inflammation. Am J Physiol 1998;274:204–8.
[5] La Cava A, Matarese G. The weight of leptin in immunity. Nat Rev Immunol 2004;4:371–9.
[6] Yilmaz Z, Ilcol YO, Ulus IH. Endotoxin increases plasma leptin and ghrelin levels in dogs. Crit Care Med 2008;36: 828–33.
[7] Wajchenberg BL, Nery M, Cunha MR, Silva ME. Adipose tissue at the crossroads in the development of the metabolic syndrome, inflammation and atherosclerosis. Arq Bras Endocrinol Metabol 2009;53:145–50.
[8] Maffei M, Fei H, Lee GH. Increased expression in adipocytes of ob RNA in mice with lesions of the hypothalamus and with mutations at the db locus. Proc Natl Acad Sci USA 1995;92: 6957–60.
[9] Masuzaki H, Ogawa Y, Sagawa N. Nonadipose tissue production of leptin: Leptin as a novel placenta-derived hormone in humans. Nat Med 1995;3:1029–33.
[10] Bado A, Levasseur S, Attoub S, Kermorgant S, Laigneau JP, Bortoluzzi MN, et al. The stomach is a source of leptin. Nature 1998;394:790–3.
[11] Sanna V, Giacomo AD, Cava AL, Lechler RI, Fontana S, Zappacosta S, et al. Leptin surge precedes onset of autoimmune encephalomyelitis and correlates with development of pathogenic T cell responses. J Clin Invest 2003;111:241–50.
[12] Gordeladze JO, Drevon CA, Syversen U. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis and mineralization: impact on differentiation markers, apoptosis and osteoclastic signaling. J Cell Biochem 2002;85:825–36.
[13] De Matteis R, Puxeddu R, Riva A, Cinti S. Intralobular ducts of human major salivary glands contain leptin and its receptor. J Anat 2002;201:363–70.
[14] Wang J, Liu R, Hawkins M, Barzilai N, Rossetti L. A nutrient-sensing pathway regulates leptin gene expression in muscle and fat. Nature 1998;393:684–8.
[15] Johnson RB, Serio FG. Leptin within healthy and diseased human gingiva. J Periodontol 2001;72:1254–7.
[16] Karthikeyan BV, Pradeep AR. Gingival crevicular fluid and serum leptin: Their relationship to periodontal health and disease. J Clin Periodontol 2007;34:467–72.
[17] Shimada Y, Komatsu Y, Ikezawa-Suzuki I, Tai H, Sugita N, Yoshie H. The effect of periodontal treatment on serum leptin, interleukin-6, and C - reactive protein. J Periodontol 2010;81:1118–23.
[18] Zimmermann GS, Bastos MF, Dias Gonçalves TE, Chambrone L, Duarte PM. Local and circulating levels of adipocytokines in obese and normal weight individuals with chronic periodontitis. J Periodontol 2013;84:624–33.
[19] Gundala R, Chava VK, Ramalingham K. Association of leptin in periodontitis and acute myocardial infarction. J Periodontol 2014;85:917–24.
[20] Söderberg S, Ahrén B, Jansson JH, Johnson O, Hallmans G, Asplund K, et al. Leptin is associated with increased risk of myocardial infarction. J Intern Med 1999;246:409–18.
[21] Leyva F, Anker SD, Egerer K, Stevenson JC, Kox WJ, Coats AJ. Hyperleptinaemia in chronic heart failure. Relationships with insulin. Eur Heart J 1998;19:1547–51.
[22] Wallace AM, McMahon AD, Packard CJ, Kelly A, Shepherd J, Gaw A, et al. Plasma leptin and the risk of cardiovascular disease in the west of Scotland coronary prevention study (WOSCOPS). Circulation 2001;104: 3052–6.
[23] Purwar P, Khan MA, Mahdi AA, Pandey S, Singh B, Dixit J, et al. Salivary and serum leptin concentrations in patients with chronic periodontitis. J Periodontol 2014; 24:1–10.
[24] WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004;363:157–63.
[25] Williams JR. The Declaration of Helsinki and public health. Bull World Health Organ 2008;86:650–2.
[26] Dudala SR, Arlappa N. An updated Prasad's socio economic status classification. Int J Res Dev Health 2013;1:26–8.
[27] Silness J, Loe H. Periodontal disease in pregnancy. Correlation between oral hygiene and periodontal condition. Acta Odontol Scand 1964;22:121–35.
[28] Loe H, Silness J. Periodontal disease in pregnancy. Prevalence and severity. Acta Odontol Scand 1963;21:533–51.
[29] Armitage GC. Development of a classification system for periodontal diseases and conditions. Ann Periodontol 1999; 4:1–6.
[30] Miller CS, Joseph DF, John TMB. Current developments in salivary diagnostics. Biomark Med 2010;4:171–89.
[31] Tonetti MS, Claffey N, European Workshop in Periodontology group C. Advances in the progression of periodontitis and proposal of definitions of a periodontitis case and disease progression for use in risk factor research. Group C consensus report of the 5th European Workshop in Periodontology. J Clin Periodontol 2005;32:210–13.
[32] Giannobile WV, Beikler T, Kinney JS, Ramseier CA, Morelli T, Wong DT. Saliva as a diagnostic tool for periodontal disease: current state and future directions. Periodontol 2000 2009;50:52–64.
[33] Chiappin S, Antonelli G, Gatti R, De Palo EF. Saliva specimen: A new laboratory tool for diagnostic and basic investigation. Clin Chim Acta 2007;383:30–40.
[34] Graves DT. The potential role of chemokines and inflammatory cytokines in periodontal disease progression. Clin Infect Dis 1999;28:482–90.
[35] Page RC. The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodontal Res 1991;26: 230–42.
[36] Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. J Periodontol 1996;67:1123–37.
[37] Arnalich F, López J, Codoceo R, Jimnez M, Madero R, Montiel C. Relationship of plasma leptin to plasma cytokines and human survivalin sepsis and septic shock. J Infect Dis 1999;80:908–11.
[38] Koh KK, Park SM, Quon MJ. Leptin and cardiovascular disease: Response to therapeutic interventions. Circulation 2008;24:3238–49.

[39] Nakata M, Yada T, Soejima N, Soejima N, Maruyama I. Leptin promotes aggregation of human platelets via the long form of its receptor. Diabetes 1999;48:426–9.
[40] Kang SM, Kwon HM, Hong BK, Kim D, Kim IJ, Choi EY, et al. Expression of leptin receptor (Ob-R) in human atherosclerotic lesions: Potential role in intimal neovascularization. Yonsei Med J 2000;41:68–75.
[41] Beltowski J, Wojcicka G, Jamroz A. Leptin decreases plasma araoxonase 1 (PON1) activity and induces oxidative stress: the possible novel mechanism for proatherogenic effect of chronic hyperleptinemia. Atherosclerosis 2003;170:21–9.
[42] Parhami F, Tintut Y, Ballard A. Leptin enhances the calcification of vascular cells: artery wall as a target of leptin. Circ Res 2001;88:954–60.
[43] Randeva H, Karteris E, Lewandowski KC, Sailesh S, O'Hare P, Hillhouse EW. Circadian rhythmicity of salivary leptin in healthy subjects. Mol Genet Metab 2003;78:229–35.
[44] Thomas T, Gori F, Khosla S, Jensen MD, Burguera B, Riggs BL. Leptin acts on human marrow stromal cells to enhance differentiation to osteoblasts and to inhibit differentiation to adipocytes. Endocrinology 1999;140:1630–8.
[45] Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, et al. Leptin regulates proinflammatory immune responses. FASEB J 1998;12:57–65.
[46] Zhao Y, Sun R, You L, Gao C, Tian Z. Expression of leptin receptors and response to leptin stimulation of human natural killer cell lines. Biochem Biophys Res Commun 2003;300:247–52.

Supplementary material available online

supplemental file.