Research Paper

Role of nonsurgical periodontal therapy on leptin levels and total antioxidant capacity in chronic generalised periodontitis patients – A clinical trial

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ARTICLE INFO

Keywords:

Leptin
Nonsurgical periodontal therapy
Periodontitis
Saliva
Total antioxidant capacity
Oxidative stress

ABSTRACT

Introduction: Periodontitis causes oxidative stress and reduce total antioxidant levels. The aim of this study was to determine the effect of non-surgical periodontal treatment on leptin levels and total antioxidant capacity in chronic periodontitis.

Materials and methods: A total of 35 chronic periodontitis (ChP) patients and 35 systemically and periodontal healthy subjects were enrolled in this study. Further, the ChP group received nonsurgical periodontal therapy (NSPT). Leptin and total antioxidant capacity (TAOC) was measured in serum and saliva samples at baseline, 3 months and 6 months after non-surgical periodontal therapy. Clinical parameters measured were measured at baseline, 1, 3 and 6 months interval.

Results: The mean serum leptin and TAOC levels in control group were significantly higher compared to chronic periodontitis group ($p < 0.05$). The control group had lower mean salivary leptin levels and higher mean salivary TAOC levels as compared to periodontitis group ($p < 0.05$). Clinical parameters were improved in ChP group post therapy ($p < 0.05$). However, the periodontal treatment showed insignificant changes in serum and salivary leptin and TAOC levels.

Conclusion: Chronic periodontitis is significantly associated with serum and salivary TAOC and leptin levels. Non surgical periodontal therapy didn't alter the local and systemic TAOC and leptin levels.

1. Introduction

Periodontitis is a chronic inflammation which causes destruction of periodontal tissues as a result of host microbial interaction. The host cells releases proinflammatory cytokines (mainly Interleukin 1 beta, Interleukin 6, tumor necrosis factor-alpha) which causes polymorphonuclear neutrophils (PMNs) migration towards inflammatory site. PMNs releases proteolytic enzymes which causes oxidative burst and release of oxygen radical known as reactive oxygen species (ROS). These ROS causes microorganisms destruction, at the same time it initiates undesirable periodontal tissue destruction.^{1,2}

Antioxidants delays or prevents oxidation of substrates, thereby provides protection to human cells against oxidative damage. In healthy

condition there lies a balance between antioxidants and oxidants, however, as oxidative stress sets in, it results in an imbalance and shift towards increase production of harmful oxidants which causes tissue destruction. Measuring the total antioxidant capacity gives us an idea of the oxidative stress and suggests role of antioxidants as interventional measures in treatment and prevention of periodontal diseases.³

Research on biomarkers to detect periodontal tissue destruction which can help in early diagnosis and treatment planning has established matrix metalloproteinase –8, 9, interleukin 1 beta as potential markers of periodontal inflammation.⁴ Certain biomarkers are specific to link periodontitis to systemic diseases, like C-reactive protein (CRP), cortisol, leptin.⁵ Depending on the severity of periodontal disease, these inflammatory mediators may show positive/negative variations as the

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<https://doi.org/10.1016/j.jobcr.2021.10.002>

Received 4 March 2021; Accepted 4 October 2021

Available online 28 October 2021

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disease progress.

Among of these leptin is a biomarker of interest as it is expressed by gingival tissues.⁶ Leptin is a nonglycosylated peptide primarily produced by adipocytes.⁷

Placenta, gastric epithelium, T-cells, osteoblasts and intralobular ducts of major salivary glands are other sources of leptin secretion.⁸ Leptin is involved in endocrine axis regulation, thermoregulation, lipid, glucose and bone metabolism, cardiovascular functions.⁹ It is also sensitive to immune inflammatory response suggesting its possible role in periodontal inflammation.¹⁰

Studies have shown lower leptin concentration in gingiva, gingival crevicular fluid (GCF) and saliva in periodontitis.^{11,12} However, higher serum leptin levels were found in serum in periodontitis patients and NSPT significantly reduced the serum leptin level.¹⁰

There are very few interventional studies investigating on possible role of leptin and total antioxidant capacity in periodontitis. In this view, the present study aimed at evaluation of local and systemic levels of leptin and total antioxidant capacity levels in patients with chronic periodontitis and its effect on periodontal treatment.

2. Materials and methods

2.1. Subjects and study design

The sample population consisted of a total of 35 subjects with ChP and 35 healthy subjects attending department of Periodontology during February 2016 to November 2016. Table 1 shows demographic characteristics of the subjects enrolled in this study. Subjects with >20 teeth present and diagnosed as ChP according to the classification by the American Academy of Periodontology,¹³ normal body mass index (BMI),¹⁴ systemically healthy were included in the study. Patients suffering from xerostomia, past history of use of mouthwash or intake of any medication in last 3 months, had received any periodontal treatment, pregnancy, lactating mother and smokers were excluded.

Written informed consent was obtained from the subjects and the study was approved by the Institutional Review Board and registered at Clinical Trials Registry-India (CTRI/2017/10/010171).

2.2. Clinical parameters measured

The clinical parameters measured were plaque index (PI),¹⁵ gingival index (GI),¹⁶ bleeding on probing (BOP),¹⁷ probing depth (PD), clinical attachment level (CAL). All the measurement was done by single examiner (SN) using UNC 15 probe (Hu-Friedy, Chicago, IL) and at six sites (mesiobuccal, distobuccal, mesiolingual, lingual and distolingual) of each tooth excluding the third molars. The intra-examiner agreement was assessed by kappa coefficient (k), which was >0.95 for all the periodontal clinical parameters.

2.3. Group 1 consisted of 35 systemically and periodontally healthy subjects

Group 2 consisted of 35 ChP subjects, having >4 mm probing depth, CAL >5 mm at 3–4 sites in more than four teeth in each quadrant with evidence of radiographic bone loss. According to recent classification

patients with Stage II Grade B periodontitis were recruited in this study.

Subjects were instructed to maintain oral hygiene throughout the study period, using soft bristle toothbrush and toothpaste.

2.4. Saliva collection

Saliva sample was collected before clinical examination to avoid blood contamination. The subjects were instructed to follow overnight fast of 8 hours, avoid brushing and intake of any food in the morning hours before sample collection. Whole unstimulated salivary sample (~5 ml) were collected in a polypropylene centrifuge tube (15 ml Falcon tube) according to splitting method. The collected sample were pipette out in an eppendorf tube and centrifuged at 4000 rotations per minute (rpm) for 10 minutes to remove the cell debris and the supernatant was stored in aliquots with code numbers at 80 °C until further analysis.

2.5. Serum collection

2 ml of blood was drawn from the ante cubital fossa by venipuncture with a 5 ml of syringe. The serum was separated from blood by centrifuging at 3000 g rpm for 5 minutes and the extracted serum was stored in aliquots with code numbers at 80 °C until further analysis.

2.6. Periodontal intervention

NSPT involved oral hygiene instructions, scaling and root planing (SRP) by ultrasonic scaler (UDS-K ultrasonic scaler (Guilin Woodpecker Medical Instrument Co. Ltd, Guilin, China) and hand gracey curettes (Hu-friedy Chicago, IL, U.S.A) and the entire procedure was completed within 2 weeks of baseline recording of clinical parameters. On the day of saliva sample collection at baseline, 3 months and 6 months interval after sample collection, before recording clinical parameters and initiation of SRP, subjects were provided snacks and the serum samples were collected after the treatment procedure. This protocol was followed throughout the study period. At 1 month completion of SRP only clinical parameters were measured.

The study timeline consisted of four visits – first one at baseline - case history, clinical parameter measurement, saliva and serum sample collection. Second visit – 1 month following SRP- oral hygiene reinforcement and clinical parameters recorded. Third and fourth visit-3 months and 6 months post NSPT, clinical parameter measured, saliva and serum samples collected (Fig. 1).

All subjects included in the study were having moderate periodontitis and any subject with progressive bone loss, > 5 mm probing depth even after 3 months of NSPT were excluded from the study and necessary treatment was provided to such subjects at periodontal surgery clinic.

2.7. Leptin assay

The leptin levels were estimated using Weldon Leptin –Elisa kit (DRG Instrument GmbH, Germany) according to the manufacturer's instructions. It was performed using quantitative sandwich enzyme immunoassay technique. The monoclonal antibody specific for leptin precoated onto the wells of the microtitre plate. Calibrated solution, serum and saliva samples were pipette into the wells and bounded to the immobilized antibody. An enzyme polyclonal antibody specific for leptin was added to the wells following washing of the unbound substance.

Elisa microreader was used for analysis using 450 nm as the primary wavelength. Comparison of samples mean optical density with the standard curve yielded the salivary concentration of leptin. The lower limit of detection was 0.5 ng/ml. The leptin level was determined in picograms (pg) and the sample concentration was calculated by dividing the leptin amount by the sample volume (pg/mL).

Table 1
Demographic parameters of different groups in the study.

Variables	Healthy controls Group A	ChP Group B
Age in years (Mean ± S.D)	46 ± 0.62	44 ± 0.84
Gender	20	20
Male	12	11
Female	8	9
BMI	20.5 ± 1.33	21.4 ± 1.42

S.D-Standard deviation, ChP-chronic periodontitis.

Fig. 1. Participants flowchart.

2.8. Total antioxidant assay

The total antioxidant assay were estimated using FRAP (Ferric Reducing Ability of Plasma) assay by Benzie and Strain.¹⁸ This method is based on the principal of ferric (Fe³⁺) reducing capacity of plasma to ferrous (Fe²⁺) ion at low pH. This in turn leads to blue coloured ferrous tripyridyltriazine (Fe²⁺ TPTZ) complex formation and absorption at 593 nm. Elisa microplate reader was used for reading and results were expressed as mmol/l (Mm/l).

2.9. Randomisation

Sample was randomised using computerised random number generator (Graphpad) in 1:1 allocation ratio. This was done by the investigator (ABS) and the subjects were enrolled in the study by clinician at the study site.

2.10. Sample size determination

The sample size was calculated based on the primary outcome of the study, two tailed unpaired *t*-test with effect size of 0.5, power of the study was 0.8 and the allocation ratio 1:1. The calculated sample size was 68 (34 subjects in each group). Attrition rate of 20% was expected, so the final sample size was 82 (41 subjects in each group). After the drop outs in each group 35 subjects were assessed.

2.11. Statistical analysis

Differences in clinical parameter, salivary and serum TAOC and leptin levels between group 1 and 2 were analyzed using the Mann-Whitney *U* test. Spearman rank correlation analysis was used to analyse correlations between clinical parameter and the leptin and TAOC levels. Intragroup differences before and after NSPT was analyzed using

Wilcoxon signed-rank test. Statistical significance was kept at $p < 0.5$. Statistical package for social science (SPSS windows version 16, Chicago, IL) was used for data analysis.

3. Results

Height and weight were recorded for evaluation of body mass index. The mean body mass index among group 1 was 20.4 ± 1.38 and in group 2 was 21.2 ± 1.44 . This difference was insignificant ($p < 0.05$) (Table 1). All the clinical parameters are illustrated in Table 2.

Leptin and TAOC were detected in all the serum and saliva samples. The control group had lower mean salivary leptin levels and higher mean salivary TAOC levels as compared to ChP group at baseline. The mean difference observed was statistically significant in both the groups ($p < 0.05$) (Tables 3 and 4). Baseline levels of mean serum leptin and TAOC levels in control group were significantly higher compared to ChP group ($p < 0.05$) (Tables 3 and 4).

The relationship between serum leptin levels and periodontal clinical parameters.

There was a significant positive correlation between serum leptin concentration and all clinical parameters which indicated decrease in leptin levels from health to periodontal disease. However, NSPT didn't show any significant change in serum leptin levels in ChP group at 3 and 6 months post therapy ($p > 0.05$) (Table 5).

The relationship between salivary leptin levels and periodontal clinical parameters.

There was a significant negative correlation between salivary leptin concentration and clinical parameters suggesting decrease in leptin levels in saliva from periodontal healthy to disease condition. Further, NSPT had no significant changes in salivary leptin levels in ChP group at 3 and 6 months post therapy ($p > 0.05$) (Table 5).

The relationship between serum TAOC levels and periodontal clinical parameters.

Table 2
Periodontal parameters among the study groups

Clinical parameters	Healthy controls	ChP	p value
	Group A Mean±S.D	Group B Mean±S.D	
PI			
baseline	0.24 ± 0.13	2.47 ± 0.32	NS
1 month	-	1.12± 0.22	<0.05
3 month	-	0.56± 0.28	<0.05
6 month	-	0.58±0.26	<0.05
p value*		<0.05	
GI			
baseline	0.42 ± 0.26	1.91 ± 0.12	NS
1 month	-	1.12± 0.13	<0.05
3 month	-	0.45± 0.13	<0.05
6 month	-	0.43±0.12	<0.05
p value*		<0.05	
PD			
baseline	2.34 ± 0.32	4.75 ± 0.43	NS
1 month	-	3.25± 0.33	<0.05
3 month	-	2.81± 0.37	<0.05
6 month	-	2.77±0.33	<0.05
p value*		<0.05	
CAL			
baseline	1.45 ± 0.12	4.07± 0.53	NS
1 month	-	3.85± 0.43	<0.05
3 month	-	2.67± 0.37	<0.05
6 month	-	2.64±0.32	<0.05
p value*		>0.05	
BoP %			
baseline	1.02 ± 0.26	4.7±0.53	NS
1 month	-	0.83±0.34	<0.05
3 month	-	0.56±0.23	<0.05
6 month	-	0.42±0.15	<0.05
p value*		<0.05	

PI-Plaque index ,GI- Gingival index ,PD- Probing depth ,CAL - clinical attachment level, BoP - bleeding on probing, ChP-chronic periodontitis , S.D-Standard deviation, p-probability value, NS- non significant , statistically significance at p<0.05.*repeated measure anova analysis for intragroup comparison.

Table 3
Serum and Saliva leptin levels.

Variable	Healthy controls Group A	ChP Group B	p value
Saliva leptin(μM)	0.29 ± 0.22	0.34 ± 0.27	<0.05
baseline	-	0.30 ± 0.24	>0.05
3 month	-	0.29 ± 0.21	>0.05
6 month	-	>0.05	
p value*			
Serum leptin(μM)	0.36 ± 0.21	0.23 ± 0.26	<0.05
baseline	-	0.24 ± 0.31	>0.05
3 month	-	0.23 ± 0.22	>0.05
6 month	-	>0.05	
p value*			

ChP-Chronic periodontitis, p-probability value, statistically significance at p < 0.05. . * Repeated measure Anova analysis for intragroup comparison.

There was a significant positive correlation between serum TAOC concentration and all clinical parameters which indicated decrease in TAOC levels from health to periodontal disease. However, NSPT didn't show any significant change in serum TAOC levels in ChP group at 3 and 6 months post therapy (p>0.05) (Table 5).

The relationship between salivary TAOC levels and periodontal clinical parameters.

There was a significant positive correlation between salivary TAOC concentration and all clinical parameters which indicated decrease in TAOC levels from health to periodontal disease. However, NSPT didn't show any significant change in salivary TAOC levels in ChP group at 3 and 6 months post therapy (p>0.05) (Table 5).

Correlation between serum and saliva leptin levels and TAOC levels.

Table 4
Serum and Saliva total antioxidant capacity levels.

Variable	Healthy controls Group A	ChP Group B	p value
Saliva	952.14 ± 32.02	543.12 ± 23.10	<0.05
TAOC (pg) baseline		567.32 ± 25.20	>0.05
3 month		546.22 ± 28.12	>0.05
6 month			
p value*			
Serum	1045.03 ± 82.23	623.18 ± 76.24	<0.05
TAOC (pg) baseline		724.12 ± 56.14	>0.05
3 month		713.16 ± 64.26	>0.05
6 month			
p value*			

TAOC - total antioxidant capacity, p-probability value, statistically significance at p < 0.05. . * Repeated measure Anova analysis for intragroup comparison.

Before the NSPT in ChP group, there was a positive correlation between serum leptin levels and TAOC levels which indicated a decrease in serum leptin and TAOC levels from health to periodontal disease. However, post therapy didn't show any significant changes at 3 and 6 months. Between the saliva leptin levels and TAOC levels before therapy, there was a negative correlations seen. Furthermore, no significant changes were seen post therapy (p>0.05) (Table 5).

4. Discussion

This study evaluated the 6 months effects of NSPT on the serum and saliva levels of leptin and total antioxidant capacity in patients with ChP. The serum leptin and TAOC levels were associated with the severity of ChP and non surgical periodontal therapy didn't have any significant changes. We tried to observe the correlation between serum and salivary leptin and TAOC levels. Several studies have reported association between leptin and periodontitis.^{19,20} Serio et al. demonstrated presence of leptin within healthy and inflamed gingiva.⁶ The leptin concentration was found highest in healthy gingiva in the solubilised gingival biopsies. Karthikeyan et al. showed positive association between GCF leptin, gingivitis and periodontitis in Indian population.²¹ Our study also found positive association of serum leptin and periodontitis. Purwar et al. studied salivary leptin levels in periodontitis and found lower salivary leptin levels in periodontitis as compared to healthy subjects.²² This is in accordance with our study, we also found significantly lower salivary leptin levels in periodontitis. However, NSPT didn't show any significant changes in either serum or salivary leptin levels.

Leptin is a peptide hormone which has a structural homolog to cytokine family and shows cytokine like expressions during inflammation.²³ It also has a role in insulin and glucose metabolism, endothelial functions and vascular tone. It acts as a proinflammatory cytokine and has synergistic action along with IL-6, IL-1 which explains the increase in leptin expression during inflammation and infections like periodontitis.²⁴ Leptin mediates local periodontal inflammation by modulating immunocytes like T-cells, monocytes and natural killer cells and has a direct stimulatory effect on bone growth through osteoblast proliferation and differentiation thereby regulating alveolar bone loss.²⁵ Thus, periodontal disease can cause changes in leptin levels locally and systemically.

In our study also we found association between serum and salivary leptin levels and periodontitis, however NSPT didn't show any improvement. This result was contradicted by Purwar et al. who found that non-surgical therapy can increase the leptin levels in the saliva of chronic periodontitis patients.²² Such variation might be due to variations in host immune response in different individuals, duration of follow up as only 12 weeks follow up was done in this study. There lies a possibility that post therapy the serum and saliva leptin levels get altered however they are not sustained in long term follow up and so in our study post 3 and 6 months no significant changes were noted.

Table 5

Spearman's Rank correlation (r) coefficient in ChP group.

		Serum leptin	Saliva leptin	PD	CA loss	Serum TAOC	Saliva TAOC
Serum leptin	Correlation Coefficient	1.000					
	Baseline						
	3 months						
	6 months						
	P value						
Saliva leptin	Correlation Coefficient	−0.346	1.000				
	Baseline	−0.312					
	3 months	−0.311					
	6 months						
	P value	<0.05					
PD	Correlation Coefficient	0.324	−0.223	1.000		0.363	–
	Baseline	0.313	−0.212			0.312	0.361
	3 months	0.324	−0.234			0.345	0.314
	6 months						0.323
	P value	<0.05	<0.05			<0.05	<0.05
CA loss	Correlation Coefficient	0.367	−0.345		1.000	0.365	−0.342
	Baseline	0.234	−0.314			0.324	−0.344
	3 months	0.213	−0.322			0.313	−0.347
	6 months						
	P value	<0.05	<0.05			<0.05	<0.05
Serum TAOC	Correlation Coefficient	0.232		0.312	0.324	1.000	
	Baseline	0.234		0.322	0.327		
	3 months	0.245		0.314	0.318		
	6 months						
	P value	<0.05		<0.05	<0.05		
Saliva TAOC	Correlation Coefficient		−0.315	−0.213	−0.245		1.000
	Baseline		−0.312	−0.234	−0.234		
	3 months		−0.322	−0.215	−0.243		
	6 months						
	P value		<0.05	<0.05	<0.05		

PD- Probing depth, CAL - clinical attachment level, TAOC - total antioxidant capacity, p-probability value, statistically significant at $p < 0.05$.

Another study by Shimada et al. on effects of periodontal treatment on serum leptin and interleukins levels post 1 month follow up after nonsurgical therapy showed decreased levels of proinflammatory interleukins and leptin levels.²⁶ Again the results are having short follow up and they found positive correlation between serum leptin levels and periodontal clinical parameters which was also observed in the present study. Gonclaves et al. found no significant effect of NSPT in circulating levels of leptin which is in accordance to our study.²⁷

Leptin in saliva has physiologic effects on oral keratinocytes, wound healing, haematopoiesis, angiogenesis, osteogenesis, immune and inflammatory reactions. It also has protective effect on gingival tissues.²⁸

Antioxidants are a substance which scavenges the free radicals released from enzymes and protects the normal cells from oxidation.²⁹ They help in maintaining a balance between antioxidant-oxidant stress. This oxidative stress can be measured by various methods and measuring each reactive oxygen species is cumbersome and expensive, so we decided to measure total antioxidant capacity as a representative of reactive oxygen species which causes oxidative stress. FRAP method was used to determine the TAOC levels. The advantage of this method is it is sensitive to blood and any contamination of samples can be

discarded. In the present study we evaluated serum and salivary TAOC levels between health and periodontitis patients. A significant difference was seen between the healthy and periodontitis group in serum and salivary TAOC levels ($p < 0.05$). However, post NSPT at 3 and 6 months no significant changes were seen at serum and saliva ($p > 0.05$). This result is similar to Sculley et al. where they found decrease salivary TAOC levels is associated with periodontitis.³⁰ Another study by Chapple et al. and Brock et al. showed decrease level of serum TAOC levels which is in agreement with the present study.^{31,32} However, post therapy at 6 and 12 months no significant changes were seen ($p > 0.05$). Similar results were seen by Kim et al. where total antioxidant status decreased directly after NSPT. With time, it increased slightly and was relatively unchanged compared to the baseline at 3 months.³³ The results of this study gives an insight that changes in antioxidant levels might be time dependent event, post intervention the levels might seem changed but over a long follow up the changes seem subtle. Akpınar et al. showed reduce serum oxidative stress post periodontal treatment which contradicts our study.³⁴ The findings also suggest that significant relationships are present between oxidant-antioxidant status and periodontal parameters in the pathology of periodontitis. However, further

studies are needed to confirm whether oxidant status is a cause of periodontitis. Such studies might lead to targeting oxidant status as a therapy for periodontitis.

The limitation of the present study is the short term follow up, individual reactive oxygen species was not measured rather total antioxidant capacity was taken as a representative of all ROS.

5. Conclusion

There lies a significant association between serum and salivary total antioxidative capacity, leptin levels and clinical parameters in chronic periodontitis patients when compared with control. Non-surgical periodontal therapy seems to restore the periodontal health, however its role in local and systemic TAOC and leptin levels remains uncertain and future multicentre longitudinal trials are needed to explore its relation.

Contribution

Swet Nisha: Concepts, Design, Literature search, Clinical studies, Experimental studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review, Avinash Bettahalli Shivamallu: Concepts, Design, Definition of intellectual content, Literature search, Data acquisition, Manuscript preparation, Manuscript review, Akila Prashant: Design, Literature search, Data analysis, Manuscript editing, Manuscript review, Manish Kumar Yadav and Sheela Kumar Gujjari-Literature search, data acquisition, manuscript editing, manuscript review, Pratibha Shashikumar: Concepts, Design, Definition of intellectual content, Manuscript editing, Manuscript review, Guarantor.

Declaration of competing interest

No conflict of interest.

Acknowledgement

Nil

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