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Effects of Non-Surgical Periodontal Therapy on Serum Leptin Levels in Chronic Periodontitis Patients - A Clinico Biochemical Study

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ABSTRACT

Background and aim: Various biomarkers in gingival crevicular fluid, saliva, and serum have been evaluated in patients with periodontitis. A Leptin concentration in serum of patients with chronic periodontitis has not been evaluated, for changes in relation to the effectiveness of periodontal therapy. So the aim of this in-vivo interventional study is to evaluate the effects of nonsurgical periodontal therapy in serum leptin levels in periodontitis patients.
Materials and methods: 24 patients participated in the study. Clinical parameters like plaque index, gingival index, modified sulcular bleeding index, probing depth and clinical attachment levels were recorded. Serum leptin level was estimated pre and 1 month post periodontal therapy. Statistical analysis was done using student t-test.
Results: All the samples from 24 patients showed positive leptin levels during assay procedure. The difference in levels of serum between healthy subjects and chronic periodontitis patients was statistically insignificant. Clinical parameters evaluation showed a significant improvement in the periodontal status after non-surgical periodontal therapy in chronic periodontitis patients.
Conclusion: It was found that serum leptin level can be used as a biomarker in evaluating the effectiveness of periodontal therapy in chronic periodontitis patients. Serum leptin levels were not severely altered in chronic periodontitis patients when compared to healthy subjects and were unable to determine the effectiveness of periodontal treatment in chronic periodontitis patients. More interventional studies with higher sample size are required to ascertain the role of serum leptin in evaluating the periodontal health status.

Keywords: Chronic periodontitis, leptin, serum, Clinical periodontal parameters, non-surgical periodontal therapy, ELISA.

INTRODUCTION

Chronic Periodontitis (CP), a chronic inflammatory disease of microbial etiology, arises from an ecological imbalance between the microbial biofilm on the teeth and the host immune inflammatory response, leading to the breakdown of tooth-supporting structures, ultimately causing tooth loss. This destructive process is mediated by the generation of cytokines, eicosanoids and matrix metalloproteinases, which in turn activate host cells, inducing significant connective tissue damage and bone breakdown.[1]

Leptin, a 16-kDanon-glycosylated peptide hormone is synthesized predominantly by adipocytes and in minor quantities by placenta, gastric epithelium, T cells, osteoblasts, and intralobular ducts of the major salivary glands.[1]Leptin can be considered a link between the neuroendocrine and immune systems because of its dual nature as a hormone and a cytokine. The increase in leptin production

that occurs during infections and inflammatory processes strongly suggests that this adipokine is part of the cytokine network that governs the inflammatory/immune response and host defense mechanisms.[1]

Currently the major limitations that prevented people from recognizing the full potential of disease detection and have seriously hampered the development of clinical diagnostics are; lack of definitive molecular biomarkers for specific diseases, lack of an easy and inexpensive sampling method with minimal discomfort and lack of an accurate, easy to use and portable platform to facilitate early disease detection.[3]

However, leptin concentrations in serum of patients with chronic periodontitis have not been explored despite the potential role of serum biomarkers in determining the presence, risk, progression and effects of therapy on periodontal disease. Hence the present investigation is done to evaluate the leptin levels in serum of chronic periodontitis patient pre and post non-surgical periodontal therapy and thereby to confirm that serum leptin levels are biomarkers for periodontal health status.

MATERIALS AND METHODS

Study design

The present investigation was conducted after taking the due clearance from the Ethical Committee of Sri Siddhartha Dental College and obtaining informed consent in the Department of Periodontics, Sri Siddhartha Dental College and Hospital, Aglakote, Tumkur and Laboratory Settings (ACUMEN LABORATORY, Bangalore).

Inclusion and exclusion criteria

A total of twenty four patients in the age group of 25 to 60 years were recruited and the subjects were divided into 2 groups.

- **Group I– Healthy subjects (Control group)** - 12 Healthy subjects not having any clinical evidence of gingivitis/periodontitis.
- **Group II –Chronic Periodontitis patients (Study group)** – 12 patients who were diagnosed with chronic periodontitis having pocket depth of 4-6mm with minimum 20 teeth present.

Inclusion Criteria

1. Subjects with age group 25-60 years who are in good general health.
2. Subjects diagnosed with chronic periodontitis.
3. Subjects with body mass index (BMI) less than 30.

Exclusion Criteria:

1. Subjects with history of any systemic diseases.
2. Subjects who were smokers and alcoholics.
3. Pregnant / lactating mothers.
4. Subjects using or used antibiotics or immune suppressants in the last 6 months.
5. Subjects who had undergone periodontal therapy (surgical/non-surgical) in past 6 months.

Serum sample collection

Before the treatment and 1 month after periodontal treatment, the serum samples were collected in the following method:

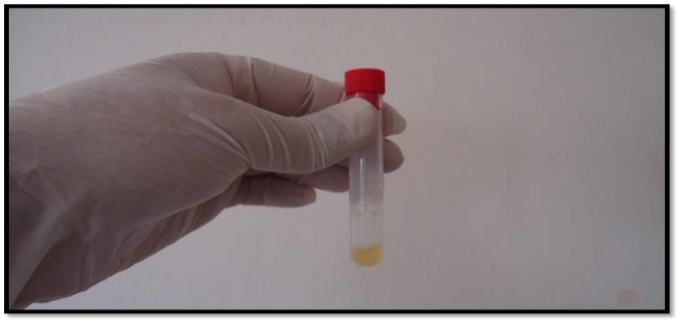

Figure 1: Serum sample collected

2 ml of blood was collected from the antecubital fossa by venipuncture. The blood sample was allowed to clot at room temperature. After 1 hour, serum was separated from blood by centrifuging at 3,000 rpm for 5 minutes, and 0.5 mL extracted serum was immediately transferred to 1.5-mL aliquots. Each aliquot was given a tracking number and sent to lab for analysis.

Group II patients were evaluated clinically after 1 month of periodontal treatment to reassess the periodontal measurements described previously.

Clinical Examination

Clinical examination was carried out by a single examiner. Periodontal health status was assessed using plaque index given by Silness and Loe (1964), gingival index given by Loe and Silness, modified sulcular bleeding index (mSBI) given by A Mombelli and associates, probing depth (PD) and clinical attachment level (CAL).

REAGENTS PREPARATION :

All reagents were brought to room temperature before use. Then 500 ml of 1 x Wash Buffer was prepared by diluting 20ml of wash Buffer concentrate 30x into deionized or distilled water. Crystals formed in the concentrate were dissolved by mixing gently.

ASSAY PROCEDURE

After preparing all reagents, standard solutions and samples as instructed, all reagents were brought to room temperature before use. The strips were inserted in the frame after determining the numbers required and the unused strips were stored in 2 to 8℃.

50 µl standard solution were added to standard well. 40 µl sample were added to sample wells and then 10 µl anti-LEP antibody were added to sample wells, then 50 µl streptavidin-HRP were added to sample wells and standard wells (Not blank control wells). Then it was mixed well and the plates covered with a sealer. Incubation was done for 60 minutes at 37℃.

The sealer was removed and the plate was washed 5 times with wash buffer. For each wash, the plates were soaked with at least 0.35 ml wash buffer for 30 seconds to 1 minute. Then the plate was bloted onto paper towels or other absorbent material.

50 µl each substrate solution A and substrate solution B were added repectively to each well and kept for incubation covered with a new sealer for 10 minutes at 37℃ in the dark. 50 µl Stop solution was added to each well and colour change of blue to yellow was noted immediately. The optical density of each well was determined using a microplate reader set to 450 nm within 30 min after adding the stop solution.

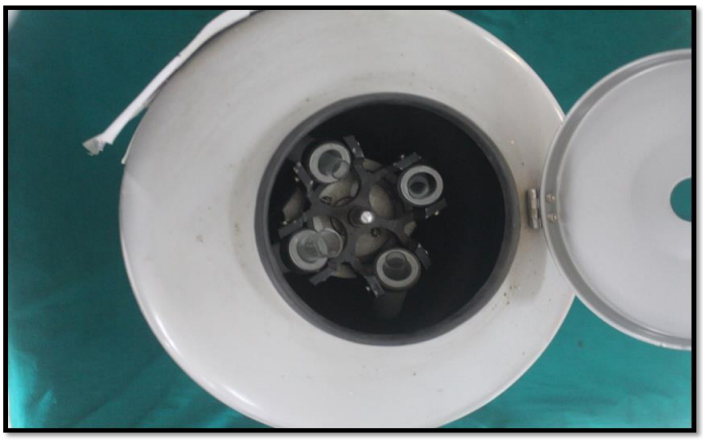

Figure 2: Centrifuging unit for sample preparation.

Figure 3: Human Leptin ELISA kit with reagents

Statistical analysis

The mean value and standard deviation were compared using student t-test and pre and post-treatment measurements were compared using student paired t-test. All statistical analysis was done under software SPSS version 16. P value ≤0.05 was considered as significant.

RESULTS

This was an in-vivo interventional study conducted to assess the effects of non-surgical periodontal therapy on serum leptin levels in chronic periodontitis patients. Subjects with normal BMI were clinically examined and divided into Group-I (healthy) and Group-II (Chronic Periodontitis patients). Along with recording of clinical periodontal parameters like plaque index (PI), gingival index (GI), modified sulcular bleeding index (mSBI), probing depth (PD) and clinical attachment level (CAL), serum was collected before and 1 month after the periodontal therapy to evaluate the leptin levels using enzyme linked immunosorbent assay.

Table 1: Comparison of characteristics of subjects between Group I (Healthy subjects) and Group II (Chronic Periodontitis patients).

Table 1 show the comparison of characteristics of subject based on gender difference between Group I (Healthy Subjects) and Group II (Chronic Periodontitis patients). It shows that mean age of males and females are 31.92 ± 5.401 and 30.00 ± 2.374 respectively, which are not statistically significant (P >0.05). It also shows that mean BMI of males and females are 26.67±1.458 and 25.93±1.861 respectively, which are not statistically significant (P >0.05).

Table 2: Comparison of characteristics of subjects in Group II (Chronic Periodontitis patients)

Table 2 shows the comparison of characteristics of subject based on gender difference in Group II (Chronic Periodontitis patients). It shows that mean age of males and females are 33.14 ± 6.842 and 30.20 ± 1.924 respectively, which are not statistically significant (P >0.05). It also shows that mean BMI of males and females are 26.03±1.694 and 25.17±0.998 respectively, which are not statistically significant (P >0.05).

Table 3: Comparison of Serum Leptin levels between Group I (Healthy subjects) and Group II (Chronic Periodontitis patients).

All the samples tested positive for the leptin assay. The mean leptin concentration in serum obtained from Group I (Healthy subjects) and Group II (Chronic Periodontitis patients) are presented in table 3. The mean leptin levels in serum were higher in Group II (Chronic Periodontitis patients) (17.55 ± 6.13 ng/ml) group than in Group I (Healthy subjects) (16.18 ± 4.11ng/ml). But the analysis of variance showed no statistically significant difference between concentrations of Leptin in Group I (Healthy subjects) and Group II (Chronic Periodontitis patients) (p >0.05).

Table 4: Comparison of Serum Leptin levels during pre and 1 month post treatment in Group II (Chronic Periodontitis patients).

All the samples in each group tested positive for leptin assay. The serum leptin levels of Group II (Chronic Periodontitis Patients) before and 1 month after treatment is showed in Table 4. The mean values of serum leptin levels before treatment were (17.55±6.13ng/ml) and 1 month after the treatment was (12.39±4.00ng/ml) and the mean difference was (5.16 ± 2.11) which was statistically significant. ($p< 0.05$).

Table 5: Comparison of clinical parameters between Group I (Healthy subjects) and Group II (Chronic Periodontitis patients).

Descriptive statistics of the periodontal parameters, plaque Index (PI), gingival Index (GI), modified sulcular bleeding index (mSBI), probing depth (PD) and clinical attachment level (CAL) at baseline of Group I (Healthy subjects) and Group II (Chronic Periodontitis patients) population are shown in Table 4.At baseline, there was differences in mean values of PI, GI, mSBI, PD and CAL between Group I (Healthy subjects) and Group II (Chronic Periodontitis patients) which was statistically highly significant ($p< 0.0001$).

Table 6: Comparison of clinical parameters during pre and 1 month post treatment in Group II (Chronic Periodontitis patients).

Table 6 show the comparison of values of plaque index (PI), Gingival index (GI), modified Sulcular Bleeding Index (mSBI), probing depth (PD) and Clinical Attachment Loss (CAL) during pretreatment and 1 month post treatment in Group II (Chronic Periodontitis patients). PI, GI, mSBI, PD and CAL decreased significantly from baseline to 1 month post treatment and the differences in their means were statistically highly significant ($p < 0.0001$).

TABLES

Table 1: Comparison of characteristics of subjects between Group I (Healthy subjects) and Group II (Chronic Periodontitis patients).

	Group I Mean ± Standard Deviation	Group II Mean ± Standard Deviation	P value	Significant
Age (Years)	31.92 ± 5.401	30.00 ± 2.374	0.2726	NS
BMI (Kg/ m^2)	25.67 ± 1.458	25.93 ± 1.861	0.7069	NS

Statistical analysis was done using unpaired t- test.
*p value ≤ 0.05 (Significant)
** pvalue ≤ 0.01 (Highly Significant)
***p value ≤ 0.0001 (Very Highly Significant)
p value >0.05 NS- Not Significant

Table 2: Comparison of characteristics of subjects in Group II (Chronic Periodontitis patients).

	Male (n=7)	Female (n=5)	P value	Significant
Age (Years)	33.14 ± 6.842	30.20 ± 1.924	0.3771	NS
BMI (Kg/m^2)	26.03 ± 1.697	25.17 ± 0.998	0.3401	NS

Statistical analysis was done using paired t- test.
*p value ≤ 0.05 (Significant)
** pvalue ≤ 0.01 (Highly Significant)
***p value ≤ 0.0001 (Very Highly Significant)
p value >0.05 NS- Not Significant

Table 3: Comparison of Serum Leptin levels between Group I (Healthy subjects) and Group II (Chronic Periodontitis patients).

	Group I Mean ± Standard Deviation (ng/ml)	Group II Mean ± Standard Deviation (ng/ml)	P Value	Significant
SERUM LEPTIN LEVEL	16.18 ± 4.11	17.55 ± 6.13	0.3596	NS

Statistical analysis was done using unpaired t- test.
*p value ≤ 0.05 (Significant)
** pvalue ≤ 0.01 (Highly Significant)
***p value ≤ 0.0001 (Very Highly Significant)
p value >0.05 NS- Not Significant

Table 4: Comparison of Serum Leptin levels during pre and 1 month post treatment in Group II (Chronic Periodontitis patients).

		Mean	Standard Deviation	P Value	Significant
SERUM LEPTIN LEVEL (nglml)	Pre treatment	17.55	6.13	0.0232*	SIG
	Post treatment	12.39	4.00		

Statistical analysis was done using paired t- test.
*p value ≤ 0.05 (Significant)
** pvalue ≤ 0.01 (Highly Significant)
***p value ≤ 0.0001 (Very Highly Significant)
p value >0.05 NS- Not Significant

Table 5: Comparison of clinical parameters between Group I (Healthy) and Group II (Chronic Periodontitis patients).

		Mean	Standard Deviation	P Value	Significant
PLAQUE INDEX	Healthy	0.7933	0.1931	<0.0001***	Highly Significant
	Chronic Periodontitis	1.373	0.1094		
GINGIVAL INDEX	Healthy	0.908	0.1247	<0.0001***	Highly Significant
	Chronic Periodontitis	1.395	0.086		
SULCUS BLEEDING INDEX	Healthy	0.9517	0.1188	<0.0001***	Highly Significant
	Chronic Periodontitis	1.478	0.093		
PROBING DEPTH	Healthy	1.521	0.20	<0.0001***	Highly Significant
	Chronic Periodontitis	5.571	0.3164		
CLINICAL ATTACHMENT LEVEL	Healthy	1.538	0.1944	<0.0001***	Highly Significant
	Chronic Periodontitis	5.664	0.2134		

Statistical analysis was done using unpaired t- test.
*p value ≤ 0.05 (Significant)
** pvalue ≤ 0.01 (Highly Significant)
***p value ≤ 0.0001 (Very Highly Significant)
p value >0.05 NS- Not Significant

Table 6: Comparison of clinical parameters during pre and 1 month post treatment in Group II (Chronic Periodontitis patients).

		Mean	Standard Deviation	P Value	Significant
PLAQUE INDEX	Pre treatment	1.373	0.1094	<0.0001***	Highly Significant
	Post treatment	1.071	0.03962		
GINGIVAL INDEX	Pre treatment	1.395	0.086	<0.0001***	Highly Significant
	Post treatment	1.060	0.025		
SULCUS BLEEDING INDEX	Pre treatment	1.478	0.093	<0.0001***	Highly Significant
	Post treatment	1.121	0.069		
PROBING DEPTH	Pre treatment	5.571	0.3164	<0.0001***	Highly Significant
	Post treatment	3.806	0.3612		
CLINICAL ATTACHMENT LEVEL	Pre treatment	5.664	0.2134	<0.0001***	Highly Significant
	Post treatment	3.927	0.2709		

Statistical analysis was done using paired t- test.
*p value ≤ 0.05 (Significant)
** pvalue ≤ 0.01 (Highly Significant)
***p value ≤ 0.0001 (Very Highly Significant)
p value >0.05 NS- Not Significant

DISCUSSION

The study concentrates on 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[3]. 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 plays a critical role in the loss of connective tissue and the supporting alveolar bone[4].

The conventional diagnostic parameters used to quantify periodontal diseases clinically include – periodontal probing depth, bleeding on probing, clinical attachment level, radiographs assessing alveolar bone level as well as various indices such as the plaque index and modified gingival index[5]. However, conventional diagnostic procedures come with limitations, one of which is that only disease history and not the current disease status, can be assessed[6].

The challenge of the biomarkers for the future lies in an earlier detection of disease progression and effectiveness of the therapy. This will drastically improve the clinical

aspect of patient management in such cases. 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[7].

The present study focuses on using Leptin as a biomarker for chronic periodontitis by evaluating altered leptin concentrations in saliva and serum among chronic periodontitis patients with normal body mass index (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 involved generalized chronic periodontitis patients and healthy volunteers.

Leptin is a 16 kDa circulating protein and polypeptide hormone which is derived from adipocytes and has been upgraded to the status of a cytokine in recent years. A popular theory put forward recently postulates that leptin manipulates the response of the host to the various inflammatory stimuli as it simultaneously increases cytokine production and phagocytosis by macrophages, inducing the production of human peripheral blood mononuclear cells and natural killer cells[8]. Leptin orchestrates numerous activities such as control of the body weight, reproduction, immune function, angiogenesis, and it's most crucial function is the regulation of inflammation[9].

According to the hallmark study done by Karthikeyan & Pradeep, that obese subjects bias the estimation of leptin level,[10] those subjects were excluded from this study by selecting only subjects with normal body mass index which was also used by the study done by Al-Maskari et al.[11] In the present study, the statistical analysis comparing the mean BMI values between the Group I (Healthy subjects) and Group II (Chronic Periodontitis patients) (Table 1) showed no statistically significant difference. This result was similar to the results obtained from the study done by Karthikeyan& Pradeep.[10]

Serum Leptin level:

In the present study the mean serum leptin concentration at baseline was found to be higher in Group II (Chronic Periodontitis patients) as compared to the Group I (Healthy subjects). The findings of the study are in accordance to the study done by Purwar et al[1], Karthikeyan et al[7], Vadvadgi et al[12], Al-Azawy et al[13] and Karam et al.[14]

The key finding of the present longitudinal interventional study reflected statistically significant reduction in serum leptin levels 1 month after nonsurgical periodontal therapy in chronic periodontitis patients, which is in accordance to the study by Shimada et al[15] and Purwar et al.[16]

10 out of 12 Group II (Chronic Periodontitis) patientstreated by non-surgical periodontal therapy showed reduction of serum leptin levels compared to baseline indicating the treatment effect may exist. The other 2 patient showed an elevation in serum leptin levels after periodontal treatment which might be due to any other unknown medical factors which have not been considered in sample selection or the inflammation might not have subsided post treatment.

The elevated serum leptin levels in chronic periodontitis patients with normal BMI could be attributed to the stimulatory action of lipopolysaccharides from periopathogens and increased levels of cytokines (TNF-a and IL-1) on adipocytes, thereby increasing the leptin production and release into the systemic circulation[17]. 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.[18] Gingiva could also contribute to the increased circulating leptin due to expression of vascular endothelial growth factor causing removal of leptin from gingiva into the circulation.[19]

The increased serum leptin levels in chronic periodontitis patients in the study before treatment may be a combinational effect of the forementioned factors.

CONCLUSION

Serum leptin levels were not severely altered in chronic periodontitis patients in contrary to healthy subjects. Non-surgical periodontal therapy of chronic periodontitis patients has altered the serum leptin levels in comparison to the baseline levels significantly and henceforth, serum leptin levels can be used as a definite biomarker in evaluating the effectiveness of periodontal therapy.

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Conflict of Interest: None

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Ethical Approval: Approved

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