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COMPARISON OF THE EFFECT OF SCALING AND ROOT PLANING ON SERUM RATIO OF LEPTIN TO ADIPONECTIN IN PATIENTS WITH MODERATE TO SEVERE CHRONIC PERIODONTITIS WITH AND WITHOUT TYPE 2 DIABETES MELLITUS.

Clinical Research

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ABSTRACT

Aim: To compare the effect of scaling and root planing on the serum ratio of leptin to adiponectin in patients with moderate to severe chronic periodontitis with and without Type 2 diabetes mellitus.

Methods: Study involved 45 subjects - Group I: 15 periodontally and systemically healthy subjects, Group II: 15 moderate to severe chronic periodontitis subjects without type 2 diabetes mellitus, Group III: 15 moderate to severe chronic periodontitis subjects diagnosed with type 2 diabetes mellitus having HbA1c levels <7%. After blood collection, periodontal parameters like plaque index, modified gingival index, pocket probing depth and clinical attachment level were recorded at baseline. Scaling and root planing was performed on Group II and Group III subjects. Periodontal parameters and serum levels of leptin and adiponectin were estimated on 45th day after scaling and root planing.

Results: At baseline the periodontal parameters like plaque index, modified gingival index, probing pocket depth, clinical attachment level and serum levels of leptin, adiponectin and leptin/adiponectin ratio were statistically significant between the groups. On 45th day, after scaling and root planing, both Group II and Group III showed a statistically significant change in periodontal parameters and serum ratio of leptin/ adiponectin.

Conclusion: Our findings suggest that leptin and adiponectin could serve as a potential biomarkers for periodontitis.

KEYWORDS

Adiponectin, Chronic periodontitis, Diabetes Mellitus, Leptin.

INTRODUCTION

Adipose tissue which is a specialized organ for fat storage and release, is considered an active endocrine gland capable of synthesizing and secreting adipokines such as adiponectin and leptin.[1] Adipokines such as leptin, adiponectin, resistin, and visfatin, a group of bioactive molecules are important in periodontal inflammation for functioning as pro-inflammatory and anti-inflammatory cytokines.

Leptin and adiponectin plays a critical role in immune response, bone and lipid metabolism, as well as in energy expenditure, and insulin sensitivity modulation. Leptin is a 16-kDa nonglycosylated peptide hormone. It is synthesized mainly in adipocytes and can regulate weight control and modulate other pathophysiological functions such as insulin sensitivity and inflammation.[2] Adiponectin, a 244-residue protein is produced mainly by white adipose tissue. Adiponectin increases fatty acid oxidation and reduces the synthesis of glucose in the liver and has anti-inflammatory properties.[3]

An increased leptin/adiponectin ratio has been shown to be related to insulin resistance in obesity, type 2 diabetes mellitus, or metabolic syndrome. Since leptin and adiponectin are also involved in the inflammatory process, this biomarker might play some role in prediction of chronic inflammation. Besides being known as an appetite controller, leptin exerts roles in both systemic and local inflammatory processes.[4]

Determining the biomarkers for screening and predicting the early onset of disease (prognostic tests) or evaluating the disease activity and efficacy of therapy (diagnostic tests) has been a great challenge in periodontology. Hence this study aims to assess the difference in serum ratio of leptin/adiponectin among moderate to severe chronic periodontitis patients with and without type 2 diabetes mellitus before and after scaling and root planing and compare them with systemically and periodontally healthy subjects.

MATERIALS AND METHODS

The subjects were recruited from the Out-patient Department of Periodontology, Meenakshi Ammal Dental College and Hospital, Chennai. A total of 45 subjects were selected and divided into three groups based on the inclusion and exclusion criteria. The study was approved by the "Institutional Review Board" MAHER - Deemed to be University, Chennai (MADC/IRB/2017/2220). The patients were explained about the study and written informed consent was obtained from those who agreed to voluntarily participate in this study.

Inclusion criteria included 1) Age group 35 to 70 years. 2)Should have ≥ 10 natural teeth. 3)Healthy controls: clinically healthy periodontium, probing pocket depth ≤ 3 mm, with no evidence of attachment loss and bone loss on radiographs, and no systemic disease. 4)Moderate to severe chronic periodontitis subjects without type 2 diabetes mellitus (HbA1c 4-6%) and signs of clinical inflammation with ≥3mm clinical attachment loss (CAL) and radiographic bone loss. 5)Moderate to severe chronic periodontitis patients (same as above mentioned criteria) with type 2 diabetes mellitus having HbA1c levels <7%. Exclusion criteria included 1) Aggressive periodontitis. 2)Smokers. 3)Pregnancy. 4) Any systemic disease like hypertension, heart disease that can alter the course of periodontal disease. 5) Those who had any course of medication affecting periodontal status within 1 month or had received periodontal therapy within 6 months. The power of the study was calculated as 98% with the sample size of 45 subjects during the course of the study.

The selected 45 subjects were divided into three groups:

GROUP I: 15 periodontally and systemically healthy subjects.

GROUP II: 15 moderate to severe chronic periodontitis subjects without type 2 diabetes mellitus.

GROUP III: 15 moderate to severe chronic periodontitis subjects diagnosed with type 2 diabetes mellitus having HbA1c levels <7%.

Subjects for all the groups were selected after a brief case history recording which included patient's chief complaint, medical and dental history, oral examination and radiographic examination. Demographic variables such as age, height and weight in healthy, moderate to severe chronic periodontitis subjects with and without type 2 diabetes mellitus were recorded in the initial visit.

5ml of fasting venous blood for the estimation of leptin and adiponectin was collected from each participant using nonanticoagulated blood tube. After 1-hour clotting at room temperature, serum was separated from blood by centrifuging at 3500 rpm for 15 minutes at 4ºC. Then, the serum was immediately transferred to an Eppendorf tube and stored at −70º C until the time of assay.

After blood collection plaque index, modified gingival index, pocket probing depth and clinical attachment level were recorded at baseline.

15 subjects in Group II and 15 subjects in Group III underwent scaling. After 7–10 days subgingival debridement was done for all the four quadrants. Oral hygiene instructions were given. Patients were re-evaluated on 45th day after completion of subgingival debridement. Blood samples were collected and periodontal parameters were recorded.

Leptin and adiponectin levels were estimated using ELISA kit (BIOASSAY TECHNOLOGY LABORATORY).

STATISTICALANALYSIS

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 20.1 The tests for normality Kolmogorov-Smirnov and Shapiro-Wilks tests revealed that all variables follow Normal distribution. To analyse the data, Parametric methods were applied. The mean values between the three groups were compared using one-way ANOVA.The linear relationship between clinical and serum parameters was assessed using Pearson correlation co-efficient. In the present study, $p<0.05$ was considered as the level of significance.

RESULTS

When Group I was compared with Group II and Group III at baseline like plaque index, modified gingival index, probing pocket depth, clinical attachment level and serum levels of leptin, adiponectin and leptin/adiponectin ratio were statistically significant. On 45th day, after scaling and root planing, both Group II and Group III showed a statistically significant change in periodontal parameters and serum ratio of leptin/ adiponectin. On inter group comparison of Group II and Group III, only modified gingival index showed a statistically significant difference. The correlation between the periodontal parameters and serum leptin level at baseline was positively correlated with all the parameters and with serum adiponectin level it was negatively correlated with all the parameters in Group II and Group III. The correlation between the periodontal parameters and serum leptin level on 45th day in Group II was negatively correlated with all the parameters and with serum adiponectin level it was positively correlated with plaque index and modified gingival index. The correlation between the periodontal parameters and serum leptin level on 45th day in Group III was negatively correlated with plaque index and modified gingival index and with serum adiponectin level it was positively correlated with plaque index and modified gingival index.

DISCUSSION

Leptin is considered to be a proinflammatory cytokine involved in the inflammatory response as it modulates the function of immunocytes such as T-cells, monocytes, and natural killer cells. These immunocytes are directly activated by leptin, leading to an elevation in the release of other inflammatory mediators.[5] Inversely, adiponectin, the insulin-sensitizing adipokine, mediates anti-inflammatory effects in the process of inflammation and functions in cell proliferation, differentiation, and regeneration.

In our study variables such as age, height and weight were recorded in the initial visit. The mean age in all the groups was above 35 years. It is seen that prevalence of chronic periodontitis is more in individuals above 35 years of age. This was consistent with the findings of Rylander et al., 1986,[6] who also reported increased prevalence of periodontitis above 35 years of age. Sheridan 1987[7] found that periodontal disease increases in prevalence and severity with age. The mean BMI in all the three groups were similar. Bahceci et al., 2007[8] established in a study that obese individuals have an increased levels of serum leptin and decreased levels of serum adiponectin. So, non-obese subjects were selected to evaluate the effect of periodontal inflammation on serum levels of leptin and adiponectin.

When the mean difference of plaque index, modified gingival index, probing pocket depth and clinical attachment level of Group I with Group II and Group I with Group III at baseline were compared, the difference was statistically significant. The mean value of plaque index, modified gingival index, probing depth and clinical attachment level were significantly higher in Group II and Group III compared to Group I. This is because Group II and Group III included subjects with moderate to severe chronic periodontitis. Group III showed a higher mean plaque index, modified gingival index, probing depth and clinical attachment level compared to Group II but the difference was statistically insignificant. In our study Group III included subjects with moderate to severe chronic periodontitis subjects with an HbA1c levels <7%. Abbas et al., 2006[9] showed a significant increase in plaque index, probing depth and attachment loss only in chronic periodontitis subjects with impaired glycemic control.

The mean value of leptin at baseline was significantly higher in Group II when compared to Group I. This is in accordance with the study done by Bullon et al., 2009[10] who stated that the increase in leptin levels in periodontitis patients is firstly due to the gingival inflammation resulting in vasodilation and secondly, serum levels of leptin would increase as a defense mechanism of the body to fight the periodontal inflammation. The mean value of leptin at baseline were higher in Group III patients when compared to Group II. This is in accordance with the study done by Ceddia RB 2002[11] who stated that serum levels of leptin are increased in type 2 diabetes mellitus. Leptin decrease pre-proinsulin mRNA expression in β cells thus decreasing the synthesis of insulin. It also reduces the release of insulin from human pancreatic β cells, which leads to the development of type 2 diabetes mellitus.

The values of adiponectin at baseline was significantly less in Group II and Group III when compared to Group I. This is in accordance with the study done by Villarreal Molina MT et al., 2012.[12] Any chronic inflammation inhibits production of adiponectin, probably through increased levels of proinflammatory cytokines. Higher adiponectin levels correlates with better insulin sensitivity and glucose metabolism and lower adiponectin levels correlates with higher insulin resistance and abnormal glucose metabolism. Lower serum adiponectin levels leading to abnormal glucose metabolism is associated with the development of type 2 diabetes mellitus. The mechanism is not very clear however, it was mentioned in the study by Durrani et al., 2013[13] that the diabetes susceptibility locus which is seen on chromosome 3q27 has been found to contain adiponectin gene and multiple polymorphisms of the adiponectin gene have also been observed in type 2 diabetes mellitus.

The comparison of values of leptin/adiponectin ratio between Group I and Group II, Group I and Group III and Group II and Group III at baseline was statistically significant (p value <0.001). Group III subjects showed significantly higher leptin/adiponectin ratio levels which shows that chronic periodontitis could influence the level of adipokines in serum and change the leptin/adiponectin ratio, and the effect would be enhanced in subjects with type 2 diabetes. Hirabara et al., 2012[14] stated that the increase in the inflammatory cytokines could impair insulin signaling pathways as well as reduce the function of mitochondria and result in insulin resistance. The ratio of leptin/adiponectin can be used as a reliable index of insulin sensitivity (Oda N et al., 2008).[15]

Studies have earlier been conducted to evaluate the serum levels of adipokines in chronic periodontitis subjects with type 2 diabetes mellitus. But the effect of scaling and root planing has not been evaluated. So in our study, after blood sample collection and baseline evaluation of periodontal parameters, Group II and Group III subjects underwent scaling and root planing. They were then recalled on 45th day to evaluate the effect of scaling and root planing (SRP) on the periodontal parameters and the serum levels of adipokines.

After scaling and root planing, both Group II and Group III showed a reduction in the plaque index and modified gingival index scores due to the reinforcement of oral hygiene. The reduction in probing pocket depth and clinical attachment level in Group II and Group III after scaling and root planing is due to decrease in inflammation following reinforcement of oral hygiene practices. The reduction in inflammatory load after scaling and root planing in Group II and Group III also led to the reduction in leptin levels. Leptin synthesis is stimulated by infection, endotoxin, and cytokines, e.g. tumor necrosis factor (TNF), leukemia inhibitory factor (LIF), and interleukin 1(IL-1) (Grunfeld C,1996).[16] Scaling and root planing leads to reduction in inflammation which reduces the levels of pro inflammatory cytokines which leads to reduction in leptin levels in Group II and Group III. Following scaling and root planing, the levels of adiponectin increased in Group II and Group III. This was in accordance with the study done by Yamaguchi et al., 2007.[17] Expression of adiponectin is regulated by pro-inflammatory mediators such as IL-6, which suppresses adiponectin transcription and translation in an adipocyte cell line (Fasshauer et al., 2003).[18]

The periodontal parameters and serum leptin, adiponectin,

leptin/adiponectin ratio were correlated using Pearson correlation co-efficient analysis. The correlation between the clinical parameters and serum levels of leptin at baseline in Group II and Group III was positively correlated and with adiponectin it was negatively correlated.

The correlation between the periodontal parameters and serum leptin level on 45th day in Group II was negatively correlated with all the parameters and with serum adiponectin level it was positively correlated with plaque index and modified gingival index. The correlation between the periodontal parameters and serum leptin level on 45th day in Group III was negatively correlated with plaque index and modified gingival index and serum adiponectin level in Group III was positively correlated with plaque index and modified gingival index. This indicates that serum leptin and adiponectin levels following scaling and root planing are more influenced by inflammation as measured by plaque index score and modified gingival index score.

Within the limitations of the study it can been inferred that chronic periodontitis could influence the level of adipokines in serum and change the ratio of leptin/ adiponectin. The serum levels of leptin and adiponectin has a potential relationship between periodontal inflammation and insulin resistance. Further studies are required to explore the mechanism and to clarify the cause-effect relationship between circulating adipokines and periodontitis.

CONCLUSION

Higher serum levels of leptin in moderate to severe chronic periodontitis subjects with and without type 2 diabetes denotes the involvement of this adipokine in the pathogenesis of periodontitis and diabetes mellitus. The lowered serum levels of adiponectin clearly shows its anti-inflammatory and anti- diabetic properties.

CONFLICTS OF INTEREST

The authors report no conflict of interest related to the study.

Table- 1 - Mean and standard deviation Serum Leptin level, Serum Adiponectin Level, Serum Leptin/Adiponectin ratio in Group I, Group II and Group III at baseline

Variable	Mean ± Std.Dev		
	Group I	Group II	Group III
Leptin (ng/ml)	2.271± 0.195	2.971± 0.253	3.459 ± 0.423
Adiponectin(mg/l)	18.310 ± 4.780	10.391±1.686	6.879 ± 2.750
Leptin/adiponectin ratio	0.134 ± 0.044	0.296 ± 0.072	0.555 ± 0.206

Table - 2 – Comparison of mean, standard deviation and test of significance of Plaque Index, Modified Gingival Index, Probing Pocket Depth (PPD), Clinical Attachment Level (CAL), Serum Leptin level, Serum Adiponectin Level, Serum Leptin/ Adiponectin ratio in Group I at base line and Group II and Group III between baseline and on 45th day.

Variable	Duration	Mean ± Std. Dev		
		Group II	Group III	Between group II and group III
Plaque Index	Baseline	2.294 0.112	2.296 0.116	
	On 45th day	0.517 0.052	0.557 0.077	
	p-value	<0.001*	<0.001*	0.108 (NS)
Modified Gingival Index	Baseline	3.155 0.161	3.190 0.0598	
	On 45th day	0.663 0.132	0.778 0.067	
	p-value	<0.001*	<0.001*	0.007*
Probing pocket depth(mm)	Baseline	6.569 0.856	6.513 0.593	
	On 45th day	4.579 0.331	4.613 0.296	
	p-value	<0.001*	<0.001*	0.765 (NS)
CAL (mm)	Baseline	6.749 0.880	6.929 0.634	
	On 45th day	4.621 0.297	4.670 0.252	
	p-value	<0.001*	<0.001*	0.632 (NS)
Leptin (ng/ml)	Baseline	2.971 0.253	3.459 0.423	
	On 45th day	2.075 0.314	2.237 0.384	
	p-value	<0.001*	<0.001*	0.215 (NS)
Adiponectin (mg/l)	Baseline	10.391 1.686	6.879 2.750	
	On 45th day	13.198 4.097	11.591 1.635	
	p-value	0.021*	<0.001*	0.169 (NS)
Leptin/ adiponectin ratio	Baseline	0.296 0.072	0.555 0.206	
	On 45th day	0.189 0.115	0.201 0.063	
	p-value	0.005*	<0.001*	0.735 (NS)

Table 3 – Correlation between clinical parameters and serum levels of leptin, adiponectin at baseline in Group II and Group III.

Variables		Group II		Group III	
		Leptin	Adiponectin	Leptin	Adiponectin
Plaque Index	Correlation	0.955	-0.892	0.886	-0.731
	P-value	0.000 *	0.000*	0.000*	0.002*
Modified Gingival Index	Correlation	0.372	-0.197	0.580	-0.360
	P-value	0.172 (NS)	0.481 (NS)	0.023*	0.188 (NS)
Probing pocket depth(mm)	Correlation	0.540	-0.699	0.619	-0.645
	P-value	0.038*	0.004*	0.014*	0.009*
Clinical attachment level (mm)	Correlation	0.571	-0.727	0.339	-0.509
	P-value	0.026*	0.002*	0.141 (NS)	0.053 (NS)

Table 4 - Correlation between clinical parameters and serum levels of leptin, adiponectin on 45th day in Group II and Group III.

Variables		Group II		Group III	
		Leptin	Adiponectin	Leptin	Adiponectin
Plaque Index	Correlation	-0.763	0.712	-0.933	0.980
	P-value	0.001*	0.003*	0.000*	0.000*
Modified Gingival Index	Correlation	-0.600	0.622	-0.723	0.831
	P-value	0.018*	0.013*	0.002*	0.000*
Probing depth(mm)	Correlation	-0.050	-0.079	0.333	-0.543
	P-value	0.859 (NS)	0.779 (NS)	0.225 (NS)	0.087 (NS)
CAL(mm)	Correlation	-0.108	-0.170	0.182	-0.347
	P-value	0.701 (NS)	0.544 (NS)	0.515 (NS)	0.206 (NS)

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