Comparative evaluation of salivary leptin levels in healthy and chronic periodontitis patients with or without Diabetes Mellitus

Running title: Salivary leptin levels in healthy and chronic periodontitis patients with or without Diabetes

ABSTRACT

INTRODUCTION: Leptin is a hormone-like protein, or some call it as an acute phase inflammatory protein, which recently has gained attention due to its role in regulating metabolism of the human body as well as affecting the body's defense mechanisms, including macrophages.

MATERIALS AND METHODS: Patients visiting Saveetha Dental College from July to November 2020 were examined. Thirty patients (15 males and 15 females) were included in this study and subdivided into three groups. Group A has participants with healthy periodontium. Group B,periodontitis and diabetes mellitus. Group C has periodontitis patients only.

RESULTS: Salivary leptin was observed to be the highest in periodontitis patients with diabetes mellitus followed by patients with periodontitis only and minimal levels in periodontal health patients.

CONCLUSION: The results indicate that salivary leptin levels are raised in the saliva of patients with periodontitis with diabetes mellitus than healthy controls and hence can be an important biomarker for periodontal therapeutic purposes.

KEYWORDS: Leptin, diabetes, periodontitis, innovative technology, inflammation.

1. INTRODUCTION

The disease of the periodontium is a chronic inflammatory condition and its severe form is characterized by periodontal ligament loss and resorption and destruction of surrounding alveolar bone [1]. It has now become one of the most common oral diseases in the world that affects many individuals eventually leading to the loss of the teeth. Previous literature has reported the prevalence of periodontitis in developing countries higher than the developed countries. Overall, the disease is prevalent with around 20-50% of the population around the world [2]. The most important risk factors that are known to be associated with periodontitis are poor oral hygiene, Diabetes, tobacco use, alcohol consumption and stress [3].

There has been consistent evidence in previous literatures regarding periodontal diseases which states diabetes mellitus is one of the major systemic risk factors which can play a role in the initiation and progression of the disease and subsequently leading to the destruction of the periodontium[4]. The Gingival crevicular fluids (GCF) and saliva of diabetic patients with periodontitis have higher levels of various types of cytokines which are inflammatory mediators as compared to non diabetic individuals with periodontitis[5]. Epidemiological studies have proved that diabetes mellitus is an important risk factor for periodontitis, and the risk of the disease is greater if glycemic control is poor. People with poorly controlled or uncontrolled diabetes are at a higher risk of periodontitis[6].

Leptin is a hormone-like protein that is derived from adipocytes that plays a major role in **mechanisms** like in metabolism, regulating weight and

function of reproductive organs. It is also known to play a significant role in certain inflammatory conditions through its direct effect on innate and adaptive immune cells [7]. Leptin is also known to have other functions such as stimulating energy expenditure, and modulation of lipid and bone metabolism, function of pancreatic beta cells, insulin sensitivity[8]. Previous literature has shown the presence of leptin in healthy gingival tissues and in the gingiva with inflammatory disease which is reduced with the progression of inflammation and is increased in pocket depth [9]. Overall, it seems that the expression of the leptin gene has an important role in the modulation of inflammatory processes such as periodontitis[10]. The present study was carried out to compare the salivary leptin levels in healthy individuals and patients with periodontitis with or without diabetes mellitus.

2.MATERIALS AND METHOD

2.1.Study design

This study was designed as a retrospective study. Patients aged 20 to 50 years, visiting Saveetha Dental hospital from July to November 2020 were examined. Thirty patients (15 males and 15 females) were included in this study and subdivided into three groups. (n for each group is not clear) Group A consisted of participants with clinically healthy periodontium. Group B with periodontitis and diabetes mellitus (type 1 or 2). Group C with periodontitis patients only.

2.1.1Inclusion criteria

Healthy periodontium of similar age and gender who had less than 10% of sites with bleeding on probing, no sites with Probing depth of 4 mm, no

clinical attachment loss >2 mm were included in group A. The inclusion criteria for periodontitis in group B and C were as follows: no more than >2 teeth missing in each quadrant; ‡30% of periodontal sites with PD ‡4 mm; ‡20% of periodontal sites with interproximal clinical AL >2 mm; ‡30% of sites showing BOP.

2.1.2 Exclusion criteria

Patients with any other systemic diseases other than diabetes mellitus, smoking habit, history of periodontal treatment in the last 6 months, betel nut users, alcoholism.

2.1.3 Saliva collection

Participants were instructed to abstain from eating, drinking, and practicing oral hygiene procedures 12 hours before saliva collection. Whole unstimulated saliva was collected from all patients by spitting into saliva containers. The samples collected were transported to the laboratory and assessed for Leptin levels using ELISA method.

2.2 METHODOLOGY

Leptin Levels (Human LEP) in saliva samples were measured using EliKine kit which is a commercially available kit that uses a quantitative sandwich enzyme immunoassay technique which has an antibody specific for Human LEP. The samples were diluted with the calibrator diluent provided with the kit in the ratio of 1:100, and the assay was performed according to the manufacturers' instructions. Standards were included in each run and all results were reported within the linearity of the assay. The average of the duplicate readings for each standard, control, and sample were noted and subtracted from the average zero standard optical density (O.D). The concentrations read from the standard curve were multiplied by the dilution factor.

The results were reported as concentration of Human LEP in picograms per milliliter of sample.

2.3 statistical analysis

Obtained results are tabulated into excel sheets and imported to Statistical Package of Social Sciences (SPSS,version 22). One way ANOVA test was used to provide statistical significance with $P<0.05$.

3.RESULTS

Among the 30 individuals, the salivary leptin levels were observed to be elevated in people with both periodontitis and diabetes mellitus than healthy periodontium individuals and people with periodontitis without diabetes mellitus.

Table 1 shows the mean ± SD salivary levels of leptin in the healthy individuals, individuals with periodontitis and diabetes and individuals with periodontitis only were 49.20±6.82, 86.20±9.93 and 64.20±6.57 pg/mL respectively. The *P* value for the three groups was found to be highly significant, $P< 0.0001$.

INTERGROUP COMPARISON	CLINICALLY HEALTHY	CP WITH DIABETES	CP
MEAN	49.20	86.20	64.20
STD.DEVIATION	6.82	9.93	6.57
P VALUE	<0.0001		

Table 1. Descriptive statistics of salivary leptin levels in healthy subjects, chronic periodontitis (CP)with diabetes and Periodontitis.

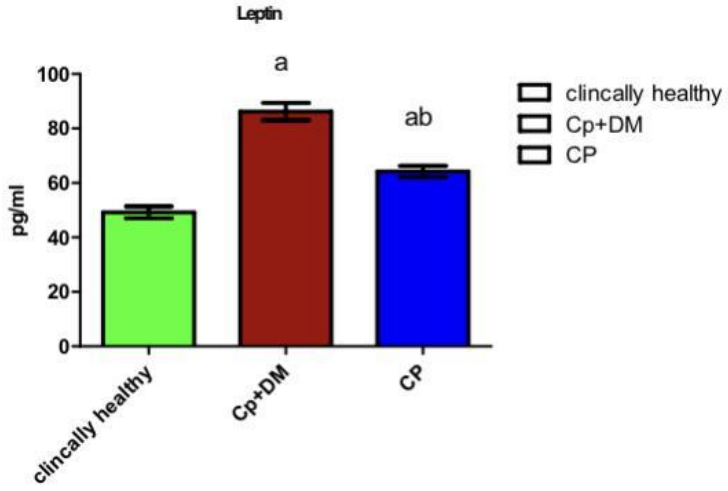

Fig. 1. Assessment of salivary Leptin concentration among periodontal health, periodontitis with diabetes mellitus and periodontitis only. The levels of salivary leptin were assessed by the Enzyme linked immunosorbent assay method. Significance at $P<0.05$($P=0.0001$), a - compared with periodontal health group. b - compared with periodontitis with diabetes mellitus.

4.DISCUSSION

Leptin is a hormone-like protein or some call it as an acute phase inflammatory protein, which recently has gained attention due to its role in regulating metabolism of the human body as well as affecting the body's defense mechanisms, including **macrophages** [11,12]. Leptin is a member of the IL-6 family and prevents a reduction in the synthesis of mucin after the activation of lipopolysaccharides thereby having a direct effect on preventing the growth of bacteria and preserving health [13].

In the present study the salivary leptin levels were determined in healthy patients and patients with periodontitis with or without diabetes mellitus[14,15]. Based on the results of the current study, there was an increase in leptin salivary levels in periodontitis patients with diabetes mellitus compared to patients with periodontitis without diabetes mellitus and healthy subjects[16, 17]. A previous study conducted by Purwar et al.[18] evaluated leptin levels in saliva and serum of patients with chronic

periodontitis and healthy controls where there was a significantly lower in healthy controls than those with periodontitis , consistent with the results of the current study[18].

In the current study, the salivary leptin levels are observed to be higher in periodontitis patients than healthy controls corroborating with Shimada et al. [19],Seteet al.[20],Purwaret al.[18],Mendoza et al.[21],Karthikeyanet al.[22],Kanoriyaet al.[23].

Salivary leptin has significant physiologic effects on oral keratinocytes which contributes to the wound healing process in the oral mucosa. Salivary leptin might have a role in the antimicrobial and anti-inflammatory property of the saliva, in association with epidermal growth factor[24]. Nokhbehsaimet al.[25] evaluated the serum levels of leptin in patients with periodontitis with and without diabetic mellitus type II where it was concluded that there was a positive correlation between serum leptin level and clinical periodontal parameters[25].

The limitation of this study was this being a unicentered cross-sectional study. Future interventional studies are needed to more strongly elucidate effect of salivary leptin levels in periodontitis patients with or without diabetes mellitus.

5. CONCLUSION

It can be concluded that diabetic patients have more periodontal tissue destruction and increased salivary leptin concentrations than non-diabetic with chronic periodontitis and patients with clinically healthy gingiva. Salivary leptin may be useful biomarkers of periodontal tissue destruction and helps in determining the pathogenesis of chronic periodontitis. In the

future, it can pave the way for developing novel treatment procedures in the therapeutic management of periodontal diseases.

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