A COMPARATIVE EVALUATION OF THE MEASUREMENT OF INTERLEUKIN-1β AS SALIVARY BIOMARKER IN PERIODONTITIS PATIENTS WITH AND WITHOUT DIABETES MELLITUS

ABSTRACT

Introduction: Periodontitis is characterized by inflammatory destruction of connective tissue, loss of periodontal attachment, and resorption of the alveolar bone. Because of its activity as an inflammatory mediator as well as a modulator of the extracellular matrix and bone, the pro-inflammatory cytokine interleukin (IL)-1β.

Aim: The study aimed to evaluate Interleukin-1β as a salivary biomarker in periodontitis patients with and without diabetes mellitus.

Materials and methods: The study included 30 patients, 15 males and 15 females, ranging in age from 30 to 60 years old. Group A: Clinically healthy, Group B: Periodontitis with diabetes mellitus, and Group C: Periodontitis without diabetes mellitus were the three groups of patients under this investigation. Expectoration into sterile bulbs was used to collect whole unstimulated saliva from all patients. Following that, collected samples are sent to the laboratory, where the amount of IL-1β, as a periodontal disease marker was determined using the ELISA sandwich technique. The data were statistically analysed using One-Way ANOVA. The Newman-Keuls multiple comparison test was used to test the significance at the levels of $p<0.05$.

Results: IL-1β level was found to be significantly higher ($p<0.05$) in periodontitis with diabetes mellitus (75 ±7 pg/L) when compared with patients with periodontitis only (45.06±7 pg/L) compared with healthy controls (36.37±5.6 pg/L).

Conclusion: From the study, it was concluded that IL-1β level was significantly increased with periodontitis patients, and patients with periodontitis and diabetes mellitus, when compared to patients with clinically healthy group. In summary, diabetes mellitus and periodontal diseases are closely associated with each other. Salivary IL-1β level can be used as a biomarker in early diagnosis of chronic periodontitis in patients with diabetes mellitus.

Keywords: Interleukin-1β, Diabetes mellitus, Innovative technology, Periodontitis, Salivary biomarker.

1. INTRODUCTION

Periodontitis is characterized by the inflammatory destruction of the connective tissue, loss of periodontal attachment, and resorption of the alveolar bone. Though bacterial pathogens were needed to initiate the disease progress, it's evident that their presence alone isn't enough to cause the rate of tissue destruction in periodontitis [1]. Pathogenesis of the periodontal disease suggests that tissue destruction can result from the change of host defense process by bacterial and host products, which stimulate the host inflammatory processes [2]. During the onset and course of periodontal disease, pro-inflammatory cytokines are a significant modulator of inflammation [3, 4]. The pro-inflammatory cytokine interleukin (IL)-1β is involved in immunological modulation as well as a variety of inflammatory responses. Because of its activity as an inflammatory mediator as well as a modulator of the extracellular matrix and bone, the pro-inflammatory IL-1β has been identified as a periodontal disease marker[5,6]. Although both isoforms of IL-

1β have similar biologic activity, IL-1β is more effective in stimulating bone resorption and is the type found more frequently in periodontitis [7].

Diabetes mellitus (DM) may be a group of metabolic disorders characterized by a high blood glucose level over a protracted period of time. Type I diabetes mellitus is an autoimmune disease whereby antigen presenting T cells selectively destroy insulin producing pancreatic β-cells. The activated T cells first invade the islets, leading to insulitis. This is followed by destruction of islets, mediated by a complex interaction between activated lymphocytes, cytokines and macrophages [8]. Apoptosis is a fundamental process involved in destruction of β-cells [9]. More recently, β-cell apoptosis shows presentation of islet antigens for disease onset. B cell derived antigens are presented by antigen presenting cells such as dendritic cells in local pancreatic-draining lymph nodes.

Both periodontitis and diabetes are widespread and complex chronic illnesses with a well-established bidirectional link [10, 11]. Diabetes is a key risk factor for periodontitis, with epidemiological studies indicating that it increases the risk threefold when compared to non-diabetic persons, especially if glycemic control is inadequate [12]. It's becoming obvious that there are interactions between the two diseases that have significant clinical consequences for dental practitioners' disease management. Periodontal treatment incorporating non-surgical periodontal therapy has been linked to improved glycemic control in diabetes patients, as well as a decrease in HbA1C. Infections from untreated periodontal disease raise blood sugar levels, making diabetes difficult to manage. For these reasons, in diabetes patients' periodontitis must be managed carefully. Our team has extensive knowledge and research experience that has translated into high quality publications.[13–25],[26–30] [31, 32] The aim of the present study was to evaluate the measurement of IL-1β as a salivary biomarker in periodontitis patients with and without diabetes mellitus.

2. MATERIALS AND METHODS

Between December 2020 and February 2021, patients aged 30 to 60 years old who visited the department of periodontics at Saveetha dental college and hospitals in Chennai, Indiawere assessed. The test groups included thirty patients (15 males, 15 females) with pre-existing moderate to severe periodontitis.

2.1. Inclusion criteria

- Not more than two teeth missing in each quadrant;
- greater than or equal to 30% of periodontal sites with periodontal depth greater than or equal to 4 mm;
- greater than or equal to 20% of periodontal sites with interproximal clinical attachment loss >2 mm;
- greater than or equal to 30% of sites showing bleeding on probing; and
- radiographic evidence of bone loss are among the periodontitis enrollment criteria.

2.2. Exclusion criteria

- Individuals who had undergone periodontal treatment in the previous 6 months;

- history of medications (antibiotics or anti-inflammatory drugs) in the previous 6 months;
- smoking or use of tobacco in any form; history of alcoholism;
- betel nut users; history of known systemic diseases that would alter the healing response of the oral tissues;
- acute periodontal conditions, such as periodontal abscess and acute necrotizing gingivitis; and
- detection of periodontitis were all excluded.

2.3. Study design

The control group consisted of ten people of similar age, race, ethnicity, and sex who had fewer than 10% of sites with bleeding on probing, no sites with periodontal depth greater than or equal to 4 mm, no clinical attachment loss greater than 2 mm, and no radiographic evidence of bone loss visible in radiographs.

Ten people with periodontitis and diabetes mellitus were chosen because they had not more than two teeth missing in each quadrant, had more than or equal to 30% of periodontal sites with periodontal depth greater than or equal to 4 mm, had more than or equal to 20% of periodontal sites with interproximal clinical attachment loss >2 mm, had more than or equal to 30% of sites with bleeding on probing, and had radiographic evidence.

2.4. **Saliva collection**

Twelve hours before saliva collection, participants were instructed to abstain from eating, drinking, and doing oral hygiene routines. Expectoration into sterile bulbs was used to collect whole unstimulated saliva from all patients. The samples were immediately placed on ice and transferred to the lab, where they were centrifuged for 10 minutes at 5,000 rpm and the clear supernatants were stored in aliquots at -70°C. Within three months of collection, the samples were thawed and the assay was carried out.

2.5. IL-1β analysis in saliva

A commercially available enzyme-linked immunosorbent assay kit that was specific for human IL-1β was used to assess IL-1β levels in saliva samples in duplicate (Figure 1). The quantitative sandwich enzyme immunoassay approach was employed in this assay. The samples were diluted in a 1:4 ratio with the calibrator diluent included in the kit, and the assay was carried out according to the manufacturer's instructions. Each run included standards, and all findings were reported within the assay's linearity. The findings of the colorimetric reaction were read directly on the automatic micro plate reader set to 450 nm as the optical density value. To achieve the actual concentration of IL-1β, the values obtained were multiplied by the dilution factor.The results were reported as concentration of IL-1β in picograms per milliliter of sample.

2.6. Statistical Analysis

The mean ± standard deviation of the triplicate analysis results of the experiments performed on control and treatment patients were expressed. The data were statistically examined using Graph Pad Prism version 5's one-way analysis of variance (ANOVA) and the Newman-Keuls multiple comparison test to determine significant differences between the mean values. The results were considered statistically significant at the 0.05 level.

Fig 1: Human IL-1beta ELISA kit

3. RESULTS AND DISCUSSION

IL-1β level was found to be significantly higher ($p<0.05$) in periodontitis with diabetes mellitus (75 ±7 pg/L) compared with periodontitis patients only (45.06±7 pg/L) and also when compared with healthy controls (36.37±5.6 pg/L) (Table 1 and Figure 2). On the basis of age and sex, individuals with periodontal health were demographically similar to patients with periodontitis with and without diabetes mellitus, although IL-1β levels were considerably different. All saliva samples from patients and controls had detectable amounts of IL-1β.

In this investigation, IL-1β was found in all samples from individuals with periodontitis, both those with and without diabetes (Table-1). Miller et al and Gursikh et al reported similar results, detecting IL-1β in all saliva samples, including those from controls [33].

In a study conducted by Teles et al, patients with periodontitis had a three-fold greater level of IL-1β [34]. Salivary IL-1β is shown to be eight-fold higher in individuals with chronic periodontitis than in healthy controls, according to Rachna Kaushik et al. There could be differences in salivary IL-1β levels due to a

variety of causes, including individual immunologic responses, the quality of local bacterial challenge, or the action of diverse combinations of inflammatory mediators, implying periodontal disease heterogeneity. Another explanation is that the local production of inflammatory mediators varies from site to site within the same patient [35].

IL-1β is a key pro-inflammatory cytokine that plays a role in periodontitis. Continuous bone loss may be produced by IL-1β, which is a potent activator of bone resorption. Increased IL-1β levels in periodontal patients with diabetes mellitus would lead to additional systemic diseases and problems, and individuals without diabetes but with periodontitis would be at risk of developing diabetes in the future [36]. Diabetes is a key risk factor for periodontitis; diabetics are three times more likely to develop periodontitis than non-diabetics. Salivary IL-1β level estimation can be employed as a non-invasive predictor for these diseases because there is a definite association between degree of hyperglycemia and severity of periodontitis.

The current study's shortcomings include a small sample size and regional restrictions. Variations in the study could be related to factors such as periodontitis stages and grading, saliva collection, and transportation. The study of salivary IL-1β in a wider population with various stages of periodontal disease and diabetes mellitus should be the focus of future research.

Table- 1: comparison of salivary IL-1β levels among 3 groups (periodontitis patients-P , patients with periodontitis along with diabetes-P+DM, and patients with periodontal health-P). The values are expressed in pg /L

Group	Periodontal health	P+DM	P	pvalue
IL-1β (pg/L)	36.37±5.6	75±7.0	45.06±7.0	P<0.0001

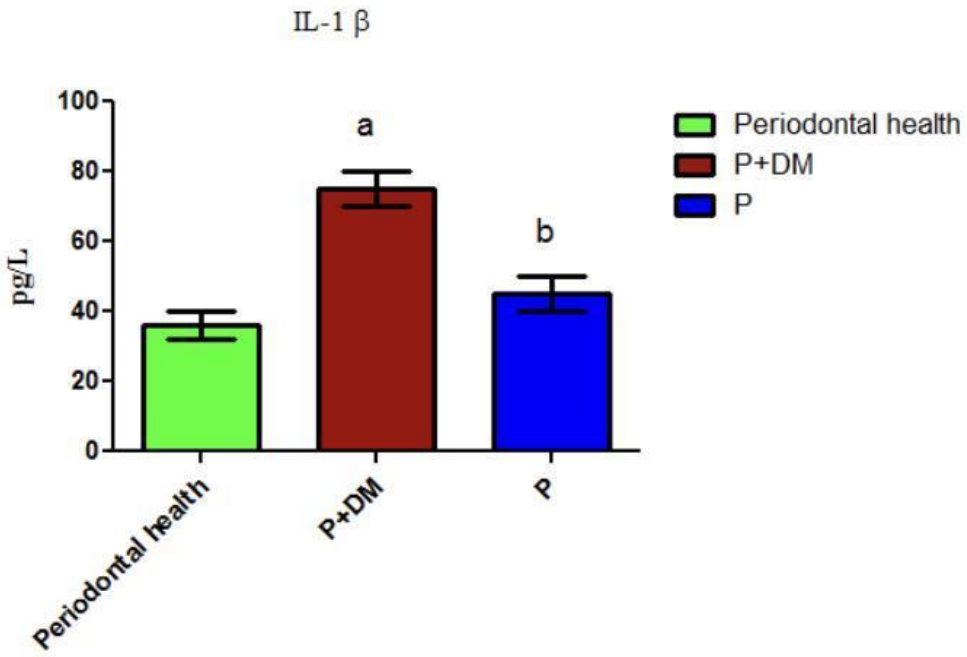

Fig 2. Assessment of salivary caspase-9 concentration among periodontal health, periodontitis and periodontitis with diabetes mellitus. The levels of salivary caspase-9 were assessed by the Enzyme Linked Immunosorbent Assay (ELISA) method. Significance at p <0.05, a- compared with the periodontal health group. b-compared with periodontitis with diabetes mellitus.

4. CONCLUSION

Within the study limitations, we concluded that IL-1β level was significantly increased in periodontitis patients and periodontitis with diabetes patients compared to periodontally healthy individuals. In conclusion, diabetes mellitus and periodontal diseases are intimately linked. IL-1β is potentially useful in distinguishing diabetes patients who are periodontally healthy and monitoring periodontal disease activity. Thus, salivary IL-1β level can be used as a biomarker in early diagnosis of periodontitis in patients with diabetes mellitus.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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