Comparative evaluation of salivary interleukin-18 in periodontitis patients with or without diabetes mellitus

ABSTRACT

Aim: The study was conducted to compare the salivary concentrations of interleukin-18 in periodontitis patients with or without diabetes mellitus

Materials and Methods: The sample size in the study was 30 and categorized into 3 groups, in each group 10 individuals were included in the respective category. The three groups are as follows: Group a- patients with clinically healthy gingiva, Group b- patients with periodontitis and diabetes mellitus, Group c- patients with periodontitis only.

Results: Salivary Interleukin-18 levels were found to be significantly higher ($p<0.013$) in periodontitis with diabetes mellitus (110±7.0 ng/L) when compared with periodontitis only (70.06±3.5 ng/L) and also when compared with healthy controls (66±5.6 ng/L).

Conclusion: From the study, diabetic patients with periodontitis have increased IL-18 levels than non-diabetic counterparts. Thus salivary IL-18 is an effective non-invasive biomarker associating periodontitis and diabetes mellitus.

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Keywords: ELISA, Innovative technology, Interleukin-18, Periodontitis, Saliva

1. INTRODUCTION

Periodontitis is a multi-factorial, chronic inflammatory disease which affects the tissues surrounding the teeth with interactions between the host defense system and the pathogenic organisms. The close relationship between diabetes (DM) and periodontitis has long been known [1]. Patients with DM are at increased risk of developing periodontitis and those with untreated periodontitis have a negatively regulated glycemic status. Possible mechanical interactions between DM and periodontitis have been suggested, including altered polymorphonuclear cell (PMN) activation, increased adipokine production and altered apoptosis which could lead to increased cytokine production in both patients with periodontitis and DM [1]. Recent studies have identified inflammation as an important factor in the pathogenesis of DM [2, 3]. In clinical studies, it was found that elevated levels of several pro-inflammatory cytokines including tumor necrosis factor-α (TNF-α), interleukin (IL) -1, IL-6, and IL-18 were associated with various types of diabetes [4 - 6].

Recent studies have reported conflicting results regarding the roles of IL-17A and IL-18 in periodontal disease. Awang et al. [7] reported significantly higher levels of IL-17A in GCF, saliva, and serum of subjects with periodontitis compared to those subjects with periodontal health. The study by Esfahrood et al. [8] did not show significant differences in both salivary and GCF levels of IL-18 between subjects with chronic periodontitis and those with a healthy periodontium. In contrast, Ozcaka et al. [9] found lower salivary IL-17A and elevated levels of IL-18 in subjects with chronic periodontitis compared with those without periodontitis. Therefore, it is still unclear whether periodontal inflammation is caused by increased levels of IL-18 and IL-17A.

In addition to the potential role of IL-17A and IL-18 in periodontitis, the systemic role of these cytokines and the interaction with DM have also been identified. In mice, DM has been shown to improve mRNA expression and IL-17 protein levels in several tissues [10,11]. In personality, however, Roohi et al. [12] revealed similar levels of IL-17A in the serum of both patients with type 1 DM and those with type 2 DM compared with healthy controls. Elevated levels of serum IL-18 have been reported in patients with metabolic syndrome and type 2 DM [13,14]. Human data on serum and salivary IL-17A and IL-18 in DM patients with and without periodontitis are still insufficient [15-25]. Our team has extensive knowledge and research experience that has translated into high quality publications [26-34]. The aim of this study is to evaluate the salivary interleukin-18 levels in periodontitis patients associated with or without diabetes mellitus.

2. MATERIALS AND METHODS

30 patients were recruited for the study and were categorized as periodontal health (Group a), patients with periodontitis and DM (Group b) and patients with periodontitis only (Group c). Unstimulated salivary samples were taken.

The patients with periodontitis were taken to the study by following the norms given below:
- not more than 2 teeth missing in each quadrant;
- greater than or equal to 30% of periodontal sites with periodontal pocket 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.
- radiographic evidence of bone loss visible in posterior bitewing films.

The patients excluded had the following features:
- patients who have undergone periodontal therapy in the last 6 months
- patients under medications like antibiotics or anti-inflammatory drugs
- patients with history of alcoholism
- patients with a history of smoking or usage of tobacco in any form, betel nut users
- patients with 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
- detection of any obvious oral mucosal lesion.

Among the 30 patients in the present study, ten individuals with clinically healthy periodontium of similar age, race, ethnicity, and sex who had <10% of sites with bleeding on probing, no sites with periodontal pocket depth greater than or equal to 4 mm, no clinical attachment loss >2 mm, and no radiographic evidence of bone loss visible in posterior bitewing radiographs formed the control group (Group a).
Ten individuals with periodontitis along with diabetes mellitus (Group b) and ten individuals with periodontitis (Group c) were selected.

2.1. Saliva Collection

Participants were instructed to refrain from eating, drinking, and practicing oral hygiene procedures 12 hours before saliva collection. Whole unstimulated saliva was collected from all patients using expectoration into sterile bulbs. Collected samples were immediately transported to the laboratory, where it was centrifuged at 5,000 rpm for 10 minutes and the clear supernatants were stored in aliquots at -70°C.

2.2 Salivary IL-18 analysis in saliva

Salivary IL-18 levels were measured in duplicate using a commercially available human IL-18 ELISA Kit procured from Abbkine Scientific Co., Ltd, China as per the manufacturer protocols. This assay is used to quantitatively analyse the IL-18 levels using sandwich enzyme immunoassay technique. The samples were diluted with calibrator diluent provided with a ratio of 1:4 and the assay was performed according to the instructions. Standards were included and all results were read as the value of optical density set to 450 nm. The intra and inter assay coefficient variance (CV) was found to be ＜11% and <9%.

2.3. Statistical analysis

The triplicate analysis results of the experiments performed on control and test subjects were expressed as mean ± standard deviation. Results were analyzed statistically by one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Newman-Keuls multiple comparison test using Graph Pad Prism version 5. The results with the $p < 0.05$ level were considered to be statistically significant.

3. RESULTS AND DISCUSSION

The present study showed that salivary Interleukin- 18 levels were significantly higher ($p<0.013$) in periodontitis with diabetes mellitus (110±7.0 ng/L) when compared with periodontitis only (70.06±3.5 ng/L) and also when compared with healthy controls (66±5.6 ng/L) (Figure 1 and Table 1). IL-18 levels are associated with both diabetes mellitus and periodontitis. Although the role of IL-18 in periodontal disease has been observed in recent studies [35,36,37], our study did show significant differences in levels of salivary IL-18 between subjects with and without type 2 DM. Ozcaka et al. [38] showed significantly higher levels of salivary IL-18 in subjects with chronic periodontitis compared to short-term healthy subjects. In addition, no elevated levels of serum IL-18 in the chronic periodontitis group were reported. In another study, plasma samples were collected from 40 patients with chronic periodontitis and 20 healthy subjects. IL-18 measurements were made using commercially available ELISA kits. Patients with chronic periodontitis showed a 46% increase in plasma IL-18 levels compared with control subjects. Notably, elevated IL-18 levels reached> 5-fold in patients with chronic periodontitis compared with healthy individuals [40]. These conflicting results between these studies may be due to the choice of subjects and the various methods used to determine IL-18 levels.

Researchers have also found an association between salivary levels IL-18 and chronic inflammation. This finding can be explained by the effect of hyperglycemic spikes on the growing inflammatory cytokines described by Esposito et al. [41]. In their study, hyperglycemic risk due to high glucose injection can increase plasma levels and can lead to increased levels of IL-6, IL-18 and TNF-α in both healthy and diabetic subjects as well. Serum IL-18 levels are associated with HbA1C but not periodontitis. Elevated levels of serum IL-18 in type 2 DM and metabolic syndrome have been reported and suggested to contribute to microangiopathy in type 2 DM in recent studies [42,43,44]. Few studies also reported an interaction between serum IL-18 and HbA1C [45, 46].

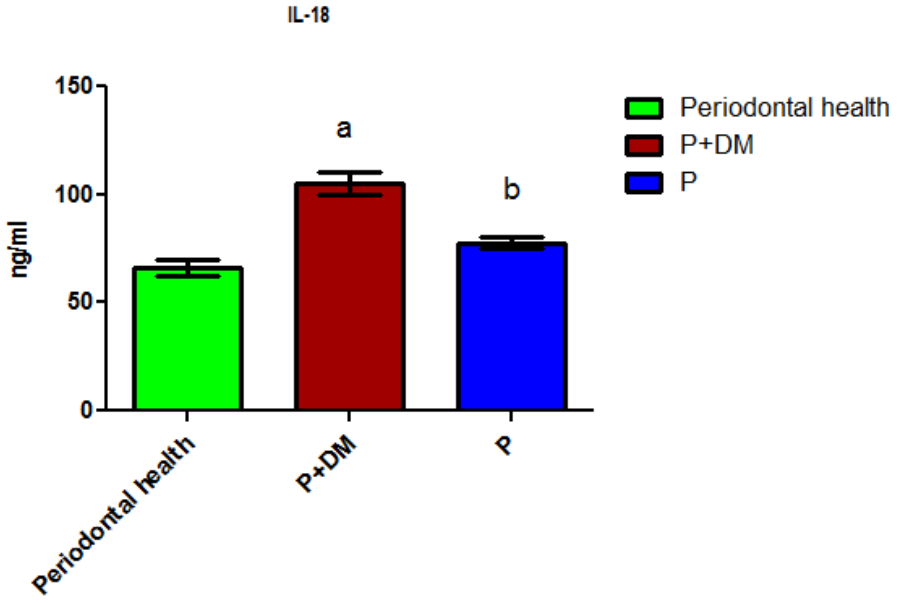

Figure 1: Assessment of salivary IL- 18 concentration among periodontal health, periodontitis and periodontitis with diabetes mellitus. The levels of salivary IL-18 were assessed by the Enzyme Linked Immunosorbent Assay (ELISA) method. Significance at $p < 0.05$, a- compared with the periodontal health group. b-compared with periodontitis with diabetes mellitus.

GROUP	PERIODONTAL HEALTH	P+DM	P	P VALUE
IL-18(ng/L)	66±5.6	110±7.0	70.06±3.5	P<0.013

Comment [a1]: In table and in graph to other designation – group a…b…c…

Table 1: Comparison of salivary interleukin 18 levels among 3 groups (periodontitis patients- P , patients with Periodontitis along with diabetes mellitus- P+DM and patients with periodontal health). The values are expressed in ng/L.

4. CONCLUSION

From the study, we conclude that IL-18 levels significantly increase in periodontitis patients with and without diabetes mellitus. In summary, diabetes mellitus and periodontal diseases are closely associated. Persistent hyperglycemia leading to exaggerated immune-inflammatory responses that are induced by periodontal pathogens is likely to be responsible for the greater risk and severity of periodontal disease in diabetic patients.

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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