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Salivary IL-1beta in diabetic patients with periodontitis, and healthy subjects with periodontitis

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A B S T R A C T

Diabetes mellitus is a systemic disease that affects more than 12 million people in the United States and represents a risk factor for periodontitis with odds ratios of 2.1 to 3.0. New data support the concept that in diabetes-associated periodontitis, the altered host inflammatory response plays a critical role. The role of IL-1β, PGE2 and MMP-3 in the pathogenesis of periodontal disease is well researched. The study was conducted to estimate the concentration of IL-1B in the saliva of diabetic and non-diabetic patients with periodontitis. The salivary IL-1B were determined in 28 patients with type II or noninsulin dependent diabetes mellitus (NIDDM) and 28 non diabetic patients with periodontitis. IL-1B was found significantly increased in diabetic patients with periodontitis, compared to non-diabetic patients. The data support the current hypothesis that the inflammation linked to periodontal disease is more severe in type 2 diabetic patients compared to the systemically healthy individuals.

Introduction

Diabetes mellitus is a major global health problem and it has an increasing prevalence due to several factors, such as population growth, aging, urbanization and increasing prevalence of obesity or lack of physical exercise. The number of people diagnosed with diabetes is increasing at an alarming rate. It is estimated that by the year 2030, 366 million people worldwide will have the disease(4) This, however, will change in the year 2025 where the greatest number of diabetic patients is expected to be from the

Asian region. (4) Diabetes mellitus is the leading cause of heart disease, stroke, hypertension, blindness and other eye problems, kidney disease, amputation and oral diseases. It had been reported in several studies that patients with T2DM have an increased risk of periodontitis (4).

Periodontal disease, a chronic infectious inflammatory disease characterized by the destruction of the tooth-supporting structures, is the most prevalent microbial diseases of mankind. It is initiated by the complex micro biota found as dental plaque, a complex microbial biofilm, and tissue destruction is largely mediated by an abnormal host response to specific bacteria and their products. Susceptibility appears to be due to a phenotype characterized by an exaggerated, "hyper-inflammatory" response to the colonizing bacteria (5).

In support of this hypothesis, some studies showed that IL-1 (Engebretson et al., 2004), IL-6 (Kurtis el al., 1999) and IL-8 (Engebretson et al., 2006) in the gingival crevicular fluid were higher in diabetic patients with periodontitis compared to healthy individuals with periodontitis. However, there is a controversy, as other studies reported similar levels of IL-1 (Kardesler et al., 2008) in patients with periodontitis with and without diabetes. Moreover, T2D was associated with an increase in IL-1β and βTG concentration in saliva, but independent of periodontal disease (Javed et al., 2012). Thus, saliva has gained attention as a source for diagnostic tests (3).

Material and Methods

Study population

This study was carried out at School of Dentistry, Tabriz University of Medical Sciences (Iran). The University Ethics Committee approved the study protocol.

The first study group included 28 T2D subjects with periodontal disease, the second one 28 systemically healthy with periodontal disease subjects. All subjects in the study groups were informed about the purpose of the study and signed an informed consent.

Inclusion and exclusion criteria

Inclusion criteria for the study groups were as follows: age over 30; ; presence of at least 20 teeth; moderate to severe periodontitis according to the criteria of the American Academy of Periodontology (Armitage, 1999); no periodontal treatment within the last six months; no antibiotic, corticoid or immunosuppressive administration within the last six months. FBS more than 126 mg/dL; (b) glycosylated hemoglobin (HbA1c) between 7.6 and 8.0% during the last 6 months.

We excluded smokers and subjects that had any inflammatory disease within the last six months.

Other additional exclusion criteria were pregnancy.

Dental examination

Clinical periodontal parameters, including plaque index, probing depth (PD), and clinical attachment level (CAL), and bleeding on probing (BOP) were assessed. A single examiner, who was trained, calibrated, and masked to the systemic condition of the patient, carried out all clinical examinations. Each tooth was measured and examined for PD in millimeters and CAL in millimeters at six sites per tooth (mesio-buccal, buccal, disto-buccal, mesio-lingual, lingual, disto-lingual)

using a Williams graduated periodontal probe. Dental plaque was scored as being present or absent at four points (mesial, buccal, lingual, and distal) on each tooth. BOP was assessed at the six sites at which PD was determined and was deemed positive if it occurred within 15 s of probing. BOP was expressed as the percentage of sites showing bleeding. Periodontal health was defined as the absence of gingival pockets ≥4 mm and absence of attachment loss ≥3 mm with no BOP. Periodontal disease was defined as two or more tooth sites with PD ≥ 4 mm or CAL of 4 mm that bled on probing.[24](antioxcidant)

We also determined blood glucose and glycated hemoglobin (HbA1c) levels for each diabetic subject, in order to evaluate the glycemic control level of diabetes mellitus.

Saliva sampling

All saliva samples were obtained in the morning after an over-night fast. The subjects were requested not to drink (except water) or chew gum for the same period and abstention was checked prior to biologic sample collection.

For the collection of saliva, the subject was seated in the coachman's position, head slightly down and was asked not to swallow or move his tongue or lips during the period of collection. Whole saliva samples were obtained by expectorating into disposable tubes prior to clinical measurements. Samples of saliva were centrifuged at 3000 rpm, 4ºC for 15 minutes, and then the clear supernatant was frozen at -80ºC until assays were performed.

Immunoassay

Salivary IL-1B levels were assessed with the ELISA-sandwich method using a commercially available immunoassay kits(human Interleukin 1B ELISA kit:E0143Hu.china) according to the manufacturer's guidelines. Results are reported in pg/ml. We also determined blood glucose and glycated hemoglobin (HbA1c) levels for each diabetic subject, in order to evaluate the glycemic control level of diabetes mellitus.

Statistical Analysis

Data were analyzed by SPSS 16.0. ANOVA. The statistical significance of associations among variables was determined by using the Spearman correlation coefficient. Statistical significance was set at $P<0.05$.

Result and Discussion

Mean age was 49. 6897 for diabetic subjects, characteristic for type 2 diabetes mellitus. The mean saliva levels of IL-1B were 0.8085 in type II diabetic patients with periodontitis (Figure 1). The mean saliva levels of IL-1B were 0.5456 in systemically health with periodontitis (Figure2). The mean of probing deapth were 4.7241 in type II diabetic patients with periodontitis but in systemically health with periodontitis were 3.6296(figure3,4).

Figure 5 shows mean of CAL in type II diabetic patients patient with periodontitis and figure 6 shows mean of CAL in systemically health with periodontitis.

Our results showed that salivary IL-1B levels in periodontal patients were higher in diabetic patients with periodontitis compared to healthy individuals with periodontitis. This might be one factor why in the presence of similar amounts of dental plaque and calculus, patients with T2D and periodontal disease had more severe periodontal problems than healthy subjects with periodontal disease.

Interleukin-1β, a pro inflammatory cytokine implicated as an effector molecule of inflammatory beta-cell destruction leading to type 1 diabetes, inhibits the function and promotes the apoptosis of beta cells. Beta cells producing interleukin-1β have been observed in pancreatic sections obtained from patients with type 2 diabetes (9).

This confirms the hypothesis that diabetes mellitus is a co-factor in the onset and evolution of periodontal disease. As a diagnostic fluid, saliva is yet insufficiently used in daily practice. It offers some advantages over serum, because of its non-invasive sampling method, which eliminates the need for clinicians' special training. Furthermore, saliva analysis yielded values of the biochemical and immunological parameters comparable with those detected from blood samples.

The concentration of IL-1β was more than the others in both of our groups, but Beklen et al showed in the macrophage/monocyte cultures of periodontitis patients that cell stimulation by IL-17 induces greater secretion of TNF-α compared to IL-1β. Dongari Bagtzoglou & Ebersole showed that the level of IL-6 concentration was higher than IL-1β. In a study carried out by Lester et al, the order of cytokine concentration in gingival samples of adult periodontitis patients was TNF-α>IL-6>IL-1β.(10)

There was significant correlation between clinical parameters (PD, CAL) and cytokine concentrations in our study,in the diabetic patient with periodontitis mean of PD and CAL is higher and Tobón-Arroyave et al[24] found a positive correlation between these parameters and IL-1β levels in salivary samples of aggressive and chronic periodontitis patients. Stashenko et al showed a significant correlation between IL-1β and CAL in adult periodontitis patients.(3)

A recent study found a relationship between clinical measures of periodontal disease and salivary levels of IL-1β and matrix metalloproteinase (MMP)-8 (Miller et al., 2006). In most of studies that have been done previously,the amount of cytokines in GCF was measured. Studies such as Javed F et al(6), Salvi et al (1), Engebretson SP, Hey-Hadavi J, Ehrhardt FJ, et al(7) and Bulut U1 et al(8) that they have been done in GCF.

Salvi el al was elevated IL-1β and PGE_2 in T1DM as compared to non-diabetes controls irrespective of level of probing depth. IL-1β and PGE_2 elevated in T1DM patients with moderate or severe chronic periodontitis as compared to patients with T1DM and gingivitis or mild chronic periodontitis

Interleukin-1beta, interleukin-6, interleukin-8, and interferon-gamma levels were higher in the presence of periodontal inflammation than in the absence of inflammation, regardless of systemic status. The interleukin-1beta and interleukin-6 levels were higher in diabetic subjects (group 3) than in systemically healthy patients with comparable types of periodontitis (group 2) (13).

Pınar Gümüş, Nurcan Buduneli show that type 1 DM patients with periodontitis exhibited significantly higher GCF levels of IL-1ß and PGE2. 25 Elevated GCF IL-1ß was associated with poor glycemic control in type 2 diabetic patients with untreated periodontitis (12)

One subject that remains to be explored is how initial periodontal therapy can influence salivary cytokines levels and glycemic control of T2D in parodontophatic patients.

Table.1 Comparison of the study variables between the two groups

	Group 1 (mean ± SD)	Group 2 (mean ± SD)
Age (years)	49.6897	45.5556
PD(mm)	4.7241	3.6296
CAL(mm)	5.2414	4.0370
Plaque index%	5.2414	42.2222

Group1: type II diabetic patients patient with periodontitis, Group2: systemically health with periodontitis

Figure.1

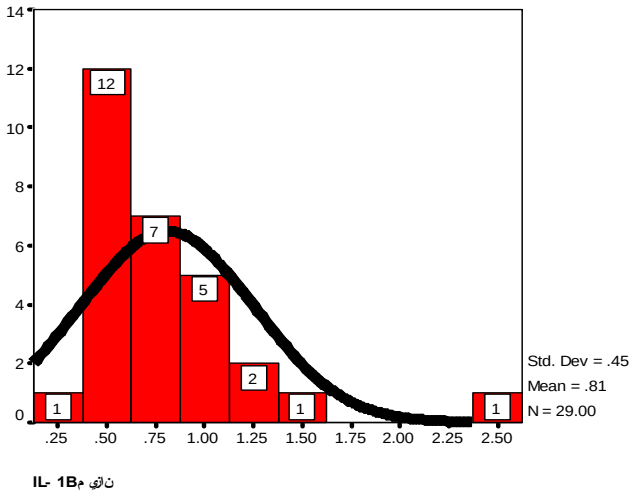


Figure.2

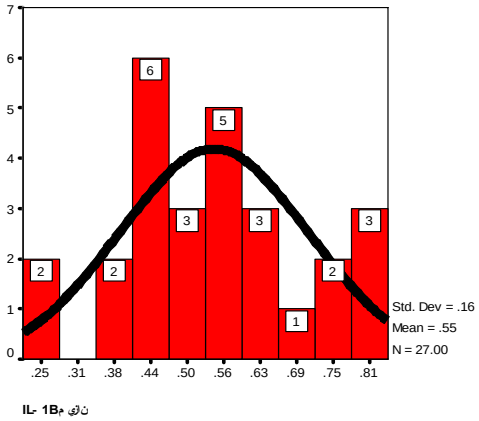


Figure.3

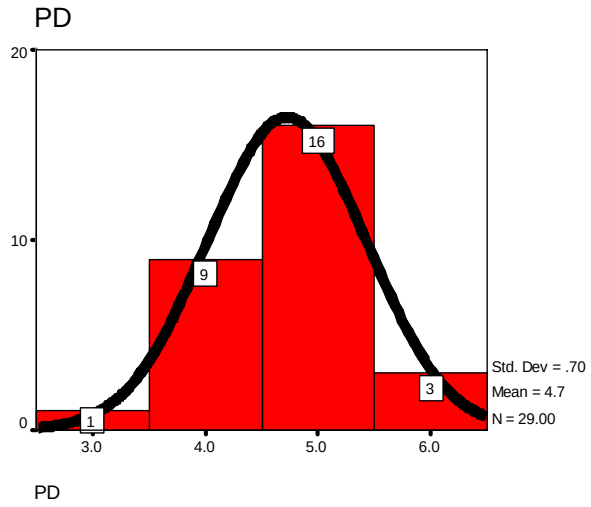


Figure.4

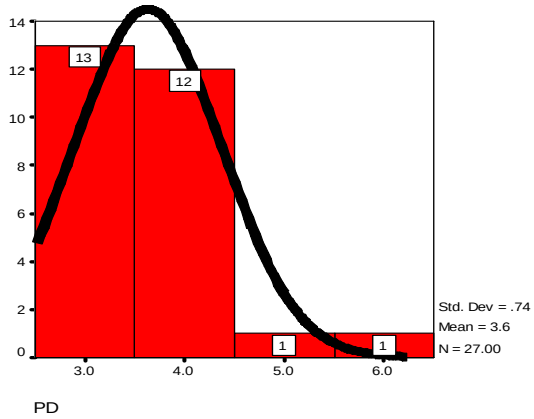


Figure.5

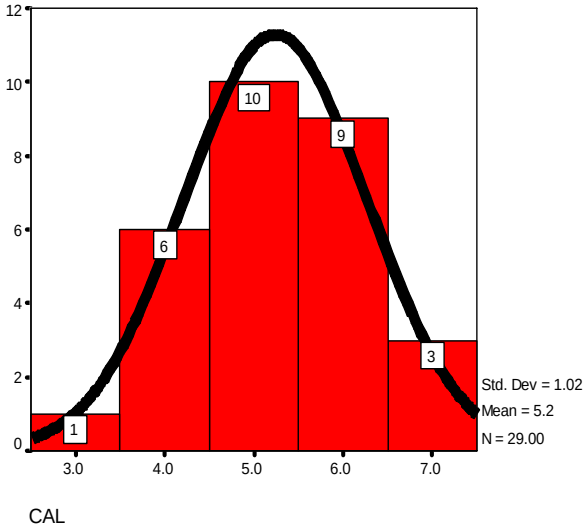
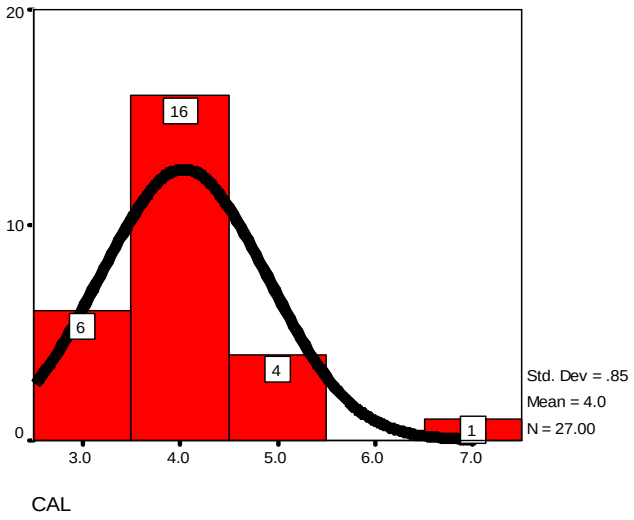


Figure.6



Conclusion

Our study showed elevated levels of Il-1B in the saliva of diabetics with periodontal disease, significantly higher than in systemically healthy subjects with periodontal disease, confirming the hypothesis that the inflammation linked to periodontal disease is more severe in type 2 diabetic patients compared to the systemically healthy individuals.

The results of our study regarding the presence of IL-1B in the saliva of diabetic patients allow us to conclude that saliva analysis is an efficient and safely enough tools for diagnosis and evaluation of periodontal disease progression in type 2 diabetic patients.

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