International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 2(G); February 2018; Page No. 10071-10077
DOI: http://dx.doi.org/10.24327/ijcar.2018.10077.1691

ResearchArticle

ASSOCIATION BETWEEN SALIVARY AND SERUM PRO-INFLAMMATORY CYTOKINES AND THE SEVERITY OF PERIODONTITIS AND GLYCEMIC CONTROL AMONG TYPE 2 DIABETIC PATIENTS

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A R T I C L E I N F O

Article History:
Received 7thNovember, 2017
Received in revised form 13th December, 2017
Accepted 3rd January, 2018
Published online 28th February, 2018

Key words:
Periodontitis, DMT2, TNF-α, IL-6, HOMA-IR

Abbreviations:
Interleukin-6 (IL-6) , Tumor Necrosis Alpha (TNF-α), diabetes mellitus type 1 (DMT1), diabetes mellitus type 2 patients (DMT2) ,glycated haemoglobin (HbA1c), homeostatic model assessment of insulin resistance (HOMA-IR), probing pocket depths (PPD), clinical attachment loss (CAL), plaque index (PI) and gingival index (GI), fasting plasma glucose (FPG),insulin resistance (IR),polymorphonuclear

A B S T R A C T

Introduction: Periodontitis is a common chronic inflammatory disease characterized by destruction of the periodontal ligament and alveolar bone. Epidemiological data confirm that diabetes is a major risk factor for periodontitis. Findings suggested that there is a bidirectional relationship between diabetes mellitus and periodontal conditions in which one can negatively affect the other. Studies suggest that salivary and serum pro-inflammatory cytokines such as Interleukin-6 (IL-6) and tumor necrosis alpha (TNF-α) are increased variably in diabetes mellitus type 2 patients (DMT2) with periodontitis compared to non-diabetic with periodontitis. Since TNF-*α* and IL-6 cytokines are believed to play a central role in inflammatory reaction and consequently alveolar bone resorption, their level in saliva and serum is believed to be significantly related to both, the degree periodontal disease and the glycemic condition in DMT2. It was also observed that the periodontal status had a significant correlation with glycated hemoglobin (HbA1c) levels of these patients.Recently several studies have revealed encouraging correlations analysis of biochemical markers of hyperglycemia, HbA1c, insulin resistance (IR), TNF-α and IL-6 with clinical parameters of periodontitis. This is considered as a remarkable diagnostic addition, dueitsearly detection and high sensitivity potentials.

Objective: The aim of the present study is to verify the hypothesis thatsalivary and serum TNF-*α* and IL-6 could be used as indicatorsfor chronic periodontitis among DMT2 patients with variable glycemic control.

Materials and methods: A total of 45 subjects were enrolled in this study.Group I; included 15well controlled DMT2patientswith moderate periodontitis but with no tooth loss, Group II; included 15 poorly controlledDMT2patientswith severe periodontitis and tooth loss, Group III; included 15 healthy cases with normal periodontium and no tooth loss.Saliva and blood samples were collected from all cases.TNF-*α* and IL-6 levels in serum and saliva were measured.IRwas alsocalculatedfor all cases by using homeostatic model assessment of insulin resistance (HOMA-IR). The HbA1c was assessed in order to evaluate the glycemic control level of the diabetic patients. The periodontal condition was assessedby radiographs and by measuring the probing pocket depths (PPD), clinical attachment loss (CAL), in addition to the plaque index (PI) andgingival index(GI) for all cases.

Results: mean values of metabolic parameters (HbAc1, fasting plasma glucose (FPG), fasting insulin and HOMA-IR) were significantly higher in the less-controlled diabetic subjects (group II) compared with well-controlled diabetics (group I) and healthy controls (group III). Mean PI was significantly higher among less-controlled diabetic subjects (group II) compared with well-controlled diabetics (group I) and healthy controls (group III) (0.46± 0.10, 0.33±0.03 and 0.32±0.05 respectively. Mean GI was significantly higher among subjects of group II compared with group III (0.31±0.06 and 0.23±0.04 respectively). Mean probing depth was significantly higher among subjects of group II compared with group I and group III (4.75±0.4, 4.25±0.35 and 2.59±0.41 respectively). Similarly mean attachment loss was significantly higher among subjects in group II compared with group I and group III (3.65±0.21, 2.77± 0.26 and 0.57± 0.03 respectively). Mean level of serum TNF-α and IL-6 were significantly higher in group II compared with group I and III (86.20±3.42, 61.66±2.52 and 5.08±0.54 respectively) and (87.50 ± 1.78, 60.00 ± 4.93 and 12.81 ± 0.41 respectively). Mean level of saliva TNF-α and IL-6 were significantly higher in group II compared with group I and III (16.95±0.74, 13.92±1.32 and 1.51±0.13 respectively) and (23.59 ± 1.13, 16.17 ± 0.93 and 5.49 ± 0.5 respectively). A strong positive correlation was found between serum and saliva TNF-α. Likewise, serum and saliva IL-6 showed a strong positive correlation.A strong positive correlation was detected between serum cytokines (TNF-α&IL-6) and metabolic parameters (HbAc1, FPG, fasting insulin and HOMA-IR). Moreover, serum cytokines showed a strong positive correlation with both probing depth and attachment loss, and a moderate positive correlation with both PI and GI.

Conclusions: Serum and saliva cytokines (TNF-*α* and IL-6)showed a strong positive correlation. In addition, a strong positive correlation was confirmed between serum cytokines (TNF-α and IL-6) and metabolic parameters (HbAc1, FPG, fasting insulin and HOMA-IR). Also a strong positive correlation was found between serum cytokines (TNF-α and IL-6) and both probing depth and attachment loss. Thus saliva and serum cytokines can be considered as indicators for chronic periodontitis activity among patients with DMT2.

INTRODUCTION

Periodontitis mainly result from local infections in the oral cavity causing irreversible destruction of the tooth attachment apparatus including; alveolar bone, root cementum, and the periodontal ligament.

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The major clinical manifestation of periodontitis is periodontal pockets. Other manifestations include redness and gingival swelling, pain, and tooth mobility. At present, periodontitis is one of the major reasons for adult tooth loss. Based on the 1999 American Association of Periodontology (AAP) classification, periodontitis can be further divided into six categories: (1) aggressive periodontitis; (2) chronic periodontitis; (3) periodontitis as a manifestation of systemic diseases; (4) necrotizing periodontal diseases; (5) periodontitis

associated with endodontic lesions; and (6) periodontitis from the developmental or acquired deformities and conditions [1].

Chronic periodontitis is one of the most common infectious public health diseases worldwide. Several studies have proven that the severity of chronic periodontitis is dependent on relations between the microbial challenge and host immune inflammatory responses [2]. Additional factors include a number of systemic diseases, especially diabetes that can exaggerate the host response to the local microbial factors resulting in destructive periodontal breakdown. [3]. Chronic periodontitis is mainly characterized by; oedema, erythema, bleeding and recession of the gingiva, in addition to tooth mobility and suppuration. It mainly results in breakdown of collagen fibers of the periodontal ligament and the development of periodontal pockets [4]. Approximately more than 10% of the population will lose a significant number of teeth as a result of chronic periodontitis [5]. Diabetes mellitus is a metabolic disorder categorized by impaired action, secretion of insulin or both, resulting in hyperglycemia. It represents with the classical symptoms; polydipsia, polyuria and polyphagia, thus diabetes mellitus is a true metabolic disorder and, thus, affects every tissue in the body [6].

Fasting plasma glucose (FPG) and 2 hours post-load plasma glucose are considered the main tests for the diagnosis of diabetes. In a patient with diagnosed diabetes, the glycated haemoglobinA1c (HbAlc) level is used to monitor the patient's overall glycemic control. HbAlc reflects the mean glucose level over the preceding 2-3 months. Thus, the interval between the 2 consecutive HbAIc tests should be at least 2 months, if any relevant changes are to be observed. This disease is classified into two main types: diabetes mellitus type 1 (DMT1) and diabetes mellitus type 2 (DMT2). DMT1 is more frequently developed in childhood due to autoimmune destruction of pancreatic β cells. DMT2 is related to disorders in insulin function and secretion and it is often associated with obesity since it promotes peripheral resistance to insulin action [7-8]. Insulin resistance (IR) is defined as the inability of peripheral tissues to adequately respond to circulating concentrations of this hormone and is commonly associated with IR[9-11]. Inflammation is characterized by local and systemic increased levels of pro-inflammatory cytokines and high infiltration of leukocytes in the inflammatory site[9,12].

Many studies have suggested a bidirectional relationship between DMT2 and periodontitis [7,9,11]. Diabetic patients have an increased risk of periodontitis, and that, once established, their periodontitis is more severe [13-14]. Research suggested that the poorer the control of diabetes mellitus is, the greater the risk of developing periodontal disease. In fact, periodontitis is recognized as the sixth complication of diabetes[3]. It is now documented that poorly controlled diabetes has a 2-9 fold increased risk of having periodontitis compared to non-diabetic subjects [15-17]. DMT2 has a remarkable effect on bone loss and coupled bone formation with higher prevalence of osteolytic lesions [18]. It was found that the function of immune cells, including neutrophils, monocytes, and macrophages, is altered in diabetes. Neutrophil adherence, chemotaxis, and phagocytosis are often impaired, which may inhibit bacterial killing in the periodontal pocket and significantly increase periodontal destruction [19]. One main reason that diabetes mellitus increases the severity of periodontal disease is that it affects negatively microvasculature beds, including soft tissues and alveolar bone supporting teeth [20]. Chronic periodontitis was found to be associated with a slight elevation in theHbA1c [21-22]. In addition, in diabetic patients, concentration of oral microbial flora is increased due to higher concentration of glucose in saliva and crevicular fluid. In literature, an enormous presence of *Staphylococcus epidermidis* and Strains of Capnocytophaga has been reported in diabetics vs non-diabetics [23]. Similarly, other studies [24-25]found that diabetics have higher than normal levels of *Prevotella intermedia*, *Prevotellamelaninogenica*, *Bacteroidesgracilis*, *Eikenellacorrodens*, *Fusobacterium nucleatum* and *Campylobacter rectus*. All the evidences regarding the biologic link between diabetes and periodontal disease support diabetes and persisting hyperglycemia leading to an exaggerated immune-inflammatory response to the periodontal pathogens [26-27] resulting in more rapid and severe periodontal tissue destruction [28].On the other hand, evidences support the concept that periodontal infection adversely affects glycemic control in people with diabetes [3]. It is confirmed that chronic periodontitis increases the risk of developing IR[15]. Studies have provided evidence that control of periodontal infection has an impact on improvement of glycemic control evidenced by a decrease in the demand of insulin and decreased HBA1c levels [20]. Cytokines are water-soluble glycoproteins secreted by hematopoietic and nonhematopoietic cells in response to infection. Their primary function is intercellular signaling (cellular communication) [29]. They also regulate immunity, inflammation, cell activation, cell migration, cell proliferation, apoptosis, and hematopoiesis. However, when cytokines are released persistently they can produce chronic diseases [30]. An inflammatory cytokine may be described as a cytokine which is induced during an inflammatory response and is associated with the onset and/or progression of the insult. Interleukin-1 alpha (IL-l α), IL-1 beta (IL-l β), IL-6, IL-8, and tumor necrosis factor alpha (TNF-α) have been categorized as inflammatory cytokines. Since alveolar bone resorption is a classical feature of periodontal disease, studies have paid particular attention to the roles of inflammatory cytokines in saliva and serum of individuals with periodontal disease [31].The increased prevalence of periodontal disease in patients with poorly controlled diabetes may be additionally explained by the role of TNF-α which plays a predominant role in inducing IR in obese subjects. It has been hypothesized that TNF-α levels in the circulation of diabetic subjects are influenced by periodontal infection and inflammation [31]. In addition, TNF-α can stimulate proliferation, multiplication and differentiation of osteoclast pioneer cells and stimulate bone resorption by indirectly activating matured osteoclastscausing periodontal tissue destruction[10]. It also activates up-regulation of inflammatory reactions and promotes mesenchymal cells to synthesize matrix metalloproteinase proteases that in turn may be related to bone resorption and destruction of the connective tissue [32]. Thus it mediates severe chronic periodontitis with severe loss of supporting structures[10]. In confirmation, periodontal treatment was found to improve the metabolic control of diabetes via improved insulin sensitivity by reducing the peripheral TNF-α concentration [31]. Similarly, it was found that IL-6 can modulate periodontal inflammation. IL-6 controls the transition from acute to chronic inflammation by changing the nature of leukocyte infiltrate from polymorphonuclear neutrophils (PMNs) to monocytes/macrophages. It also exerts stimulatory effects on T and B cells and induces antibody formation, favoring a chronic inflammatory response [31,33]. IL-6 also plays a role in fighting infection, as it has been shown to be required for resistance against some types of bacteria [34]. In addition, osteoblasts secrete IL-6 to stimulate osteoclast formation[35].

For decades, clinical diagnosis of chronic periodontitis was based on traditional diagnostic procedures such as radiographic assessment, clinical attachment loss (CAL), PPD and bleeding indices[36]. Albeit easy to use, cost-effective and relatively non-invasive, CAL evaluation using the periodontal probe requires a 2-3 mm threshold change before a site with significant breakdown can be identified[37]. Many studies have been carried out looking at the innovation of diagnosis methods whose advantages could be early detection, a non-invasive approach, high sensitivity and specificity. They revealed clear correlations analysis of biochemical markers for hyperglycemia, HbA1c, IR, TNF-α and IL-6 with clinical parameters of chronic

periodontitis [38-40]. This was a remarkable diagnostic addition. At present, saliva represents an increasingly useful auxiliary means of diagnosis [41]. Many analyses associated with periodontitis have been detected in saliva such as cytokines that can provide potentially important information regarding periodontal status [24]. TNF-α would be useful as a traceable salivary inflammatory mediator whose levels could be associated with the disease severity, showing its progression. Nonetheless, being present in the oral fluids early, even before the periodontal clinical parameters being present, is a remarkable feature that makes possible the cytokine-based diagnosis of periodontal diseases [37,42]. Recently,saliva and serum analysis of diabetic patients were introduced as efficient and safely enough tool for diagnosis and evaluation of periodontal disease progression, especially in DMT2 patients. Because serum components of saliva are derived primarily from the local vasculature that originates from the carotid arteries, saliva has a prodigious fluid source that provides many, if not most, of the same molecules found in the systemic circulation. Thus, researchers involved in periodontal disease diagnostics are seriously investigating the possible use of saliva, for disease.

The aim of the present study is to validate cytokine-based diagnosis (TNF-α & IL-6) of periodontal diseases in DMT2 cases and correlate this to the degree of glycemic control and the severity of the periodontal disease as an approach to test the hypothesis that TNF-α and IL-6 may be valuable in planning effective early prevention and treatment for subjects with altered glucose homeostasis in DMT2.

MATERIALS AND METHODS

Study population

A total of 45 male subjects were included in this study from those attending the College of Dentistry, Qassim University dental clinics. Their ages ranged between 26 and 48. All subjects in the study groups were educated 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 18; no major diabetic complications; presence of at least 20 teeth; moderate to severe periodontitis according to the criteria of the American Academy of Periodontology [1].We excluded smokers and subjects that had periodontal treatment or any systemic diseases rather than diabetes or under any medication during the previous 6 months. Obese subjects were excluded to omit the influence of obesity on IR status. Other additional exclusion criteria were: pregnancy or lactation, bleeding disorders and menstruation.

Clinical and Radiographic Examinations

Clinical examination was done using a specific algorithm, by registration of personal data, anamnesis information, extra- and intraoral examination. A single examiner carried out full-mouth periodontal parameters for all subjects. Periodontal status was assessed by measuring specific indices such as plaque index (PI) (Silness&Loe, 1964), gingival index (GI) (Loe & Silness, 1963), PPD and CAL (Christgau *et al*., 1998). PPD and CAL were recorded at six sites for each tooth (except the third molars).

Population Grouping

Group I included 15 well controlled DMT2 patients with moderate periodontitis but with no teeth loss, Group II included 15 poorly controlled DMT2 patients with severe periodontitis and teeth loss, while Group III included 15 healthy subjects with normal periodontium and no teeth loss, whom will serve as a control group.

Glycemic Status of DMT2 patients

Group I (well controlled) had their FPG around normal (126 mg/dL) and HbA1c below 6.5%, while Group II (poorly controlled) had their FPG above 126 mg/dL) and HbA1c 6.5% or more.

Samples collection

Saliva samples

Whole unstimulated salivary samples were collected between 9:00 and 10:00 a.m., before periodontal examination, using standard techniques. Briefly, subjectsrefrained from eating, drinking, and using chewing gum, for at least 1 hour prior to evaluation. Samples were obtained by requesting subjects to initially gargle for 5 min, and then subjects were asked to spit saliva into sterile 50mL centrifuge tubes for 5 min without swallowing. The tubes were cooled in ice water at all times. All samples were immediately centrifuged at 6000gfor 20 min, at 4°C, to remove cellular debris. The clarified supernatant was filtered through a low protein binding membrane, separated in polyethylene recipients, then marked for identification and stored in the refrigerator at −80°C for subsequent analysis [43].

Blood Samples

Venous blood samples were obtained by venipuncture using an adequate closed system sample. Tubes were then centrifuged at 3,000 rpm/10 min then sera was separated and stored at -20°C until analyzed.

Biochemistry analysis

Salivary and serum TNF- α and IL-6 levels were assessed with the ELISA sandwich method using a commercially available immunoassay kits (OptEIA human TNF- α, Pharmingen, USA and DuoSet ELISA Development System, R&D Systems, USA, respectively) according to the manufacturer's guidelines. Results are reported in pg/ml. We also determined FPG and HbA1c levels for each diabetic subject, in order to evaluate the glycemic control level of diabetes mellitus [44]. Fasting insulin levels were measured using commercially available assay of Mercodia Ultrasensitive human Insulin ELISA kit (AB Uppsala, Sweden). IR was also calculated by using homeostatic model assessment of insulin resistance (HOMA-IR) as follows: Fasting insulin (mUI/L) × fasting glucose (mmol/L) / 22.5 [45-46].

Statistical analysis

Data were analyzed and tabulated using statistical package for social sciences (SPSS) ver. 20 windows. After applying the test of normality, the data was found to be normally distributed, thus parametric tests were applied. The results were statistically analyzed using the one-way ANOVA test and Post-Hoc Turkey test for comparison between groups and Spearman's test to detect the correlation in study groups. Statistical significance was set at $p < 0.05$

RESULTS

Table1 shows metabolic parameters and periodontal measurements of subjects in study groups. Mean ages of subjects in group I, II and III showed no significant differences (40±7, 41±5 and 38± 9 (years) respectively) where ANOVA=0.67 and P=0.51. On the other hand, mean

values of metabolic parameters (HbAc1, FPG, fasting insulin and HOMA-IR) were significantly higher in the less-controlled diabetic subjects (group II) compared with well-controlled diabetics (group I) and healthy controls (group III) where P=0.000.

Concerning periodontal measurements; mean PI was significantly higher among less-controlled diabetic subjects (group II) compared withwell-controlled diabetics (group I) and healthy controls (group III) (0.46± 0.10, 0.33±0.03 and 0.32±0.05 respectively) but no significance was recorded between group I and III. Mean GI was significantly higher among subjects of group II compared with group III (0.31±0.06 and 0.23±0.04 respectively) but no significant differences were recorded between group I and II (0.27±0.04 and 0.31+0.06 respectively) or between group I and III (0.27±0.04 and 0.23± 0.004 respectively). Mean probing depth was significantly higher among subjects of group II compared with group I and group III (4.75±0.4, 4.25±0.35 and 2.59±0.41 respectively). Similarly mean attachment loss was significantly higher among subjects in group II compared with group I and group III (3.65±0.21, 2.77± 0.26 and 0.57± 0.03 respectively) where P=0.00

Table1: Metabolic parameters and periodontal measurements of subjects in study groups

Parameters	Group I Mean ± SD	Group II Mean ± SD	Group III Mean ± SD	ANOVA	P-Value
Age (Years)	40 ± 7	41 ± 5	38 ± 9	0.67	0.51 $(1,2)^{NS}$ $(1,3)^{NS}$ $(2,3)^{NS}$
HbAc1 (DCCT %)	6.12 ± 0.45	7.25 ± 0.87	4.71 ± 0.42	64.16	0.000 $(1,2)^{*}$ $(1,3)^{*}$ $(2,3)^{*}$
FPG (mg/dl)	109.07 ± 11.76	143.53 ± 7.5	92.93 ± 11.51	91.92	0.000 $(1,2)^{*}$ $(1,3)^{*}$ $(2,3)^{*}$
Fasting Insulin (mIU/L)	19.13 ± 3.07	28.4 ± 3	6.09 ± 0.51	302.5	0.000 $(1,2)^{*}$ $(1,3)^{*}$ $(2,3)^{*}$
HOMA-IR (mmol/L)	5.16 ± 1.2	10.07 ± 1.24	1.39 ± 0.27	297	0.000 $(1,2)^{*}$ $(1,3)^{*}$ $(2,3)^{*}$
PI (Score)	0.33 ± 0.03	0.46 ± 0.1	0.32 ± 0.05	20.48	0.001 $(1,2)^{*}$ $(1,3)^{NS}$ $(2,3)^{*}$
GI (Score)	0.27 ± 0.04	0.31 ± 0.06	0.23 ± 0.04	10.58	0.001 $(1,2)^{NS}$ $(1,3)^{NS}$ $(2,3)^{*}$
PPD (mm)	4.25 ± 0.35	4.75 ± 0.4	2.59 ± 0.41	127.1	0.000 $(1,2)^{*}$ $(1,3)^{*}$ $(2,3)^{*}$
Attachment loss (mm)	2.77 ± 0.26	3.65 ± 0.21	0.57 ± 0.03	1005.8	0.000 $(1,2)^{*}$ $(1,3)^{*}$ $(2,3)^{*}$

significant at p<0.05 ^{NS}: Non-significant.

Table 2 shows mean serum cytokines (TNF-α and IL_6) of subjects in study groups. Mean level of serum TNF-α was significantly higher in group II compared with group I and III (86.20±3.42, 61.66±2.52 and 5.08±0.54 respectively) where P=0.001. Similarly, mean level of serum IL-6 was significantly higher in group II compared with group I and III (87.50 ± 1.78, 60.00 ± 4.93 and 12.81 ± 0.41 respectively), where P=0.001

Table 2 Mean serum cytokines of subjects in study group

Serum cytokines	Group I mean ±SD	Group II mean ±SD	Group III mean ±SD	P for ANOVA
TNF-α	61.67 ±2.53	86.20 ± 3.43	5.08 ± 0.54	<0.001*
IL-6	60.00 ± 4.93	87.50 ± 1.78	12.81 ± 0.41	<0.001*

Table 3 shows mean saliva cytokines (TNF-α and IL-6) of subjects in study groups.Mean level of saliva TNF-α was significantly higher in group II compared with group I and III (16.95±0.74, 13.92±1.32 and 1.51±0.13 respectively). Similarly, mean level of saliva IL-6 was significantly higher in group II compared with group I and III (23.59 ± 1.13, 16.17 ± 0.93 and 5.49 ± 0.5 respectively) where P=0.001

Table 3 Mean salivary cytokines of subjects in study groups

Salivary cytokines	Group I mean ± SD	Group II mean ± SD	Group III mean ± SD	P for ANOVA
TNF-α	13.92 ± 1.33	16.95 ± 0.74	1.51 ± 0.13	P<0.001*
IL-6	16.17 ± 0.93	23.59 ± 1.13	5.49 ± 0.5	P<0.001*

Table 4 shows the correlation between serum and saliva cytokines (TNF-α and IL-6). A strong positive correlation was found between serum and saliva TNF-α, where r=0.990, p=0.000. Likewise, serum and saliva IL-6 showed a strong positive correlation where r=0.993, p=0.000

Table 4 Correlation between serum and salivary cytokines

Parameters	Salivary TNF- α		Salivary IL-6	
Serum TNF- α	r = 0.990	p=0.000		
Serum IL-6			r= 0.993	p= 0.000

Table 5 portrays the correlation between serum cytokines and metabolic parameters. There was a strong positive correlation between serum TNF-α and metabolic parameters (HbAc1, FPG, fasting insulin and HOMA-IR), where r= 0.855, 0.825, 0.973 and 0.934 respectively. Similarly serum IL-6 showed as strong positive correlation with metabolic parameters (HbAc1, FPG, fasting insulin and HOMA-IR) where r= 0.852, 0.856, 0.974 and 0.949 respectively

Table 5: Correlation between serum cytokines and metabolic parameters

Metabolic parameters	Serum TNF-α		Serum IL-6	
	Pearson's r	p-value	Pearson's r	p-value
HbAc1 (DCCT %)	0.855	0.000	0.852	0.000
FPG (mg/dl)	0.825	0.000	0.856	0.000
Fasting Insulin (mIU/L)	0.973	0.000	0.974	0.000
HOMA-IR (mmol/L)	0.934	0.000	0.949	0.000

Table 6 shows the correlation between serum cytokines and periodontal measurements. Serum TNF-α showed a moderate positive correlation with both PI and GI where r= 0.601 and 0.614 respectively. Yet a strong correlation was confirmed between serum TNF-αand both probing depth and attachment loss where r=0.942 and 0.955 respectively. Similarly, serum IL-6 showed a moderate positive correlation with both PI and GI where r=0.602 and 0.605 respectively, and a strong correlation was confirmed between serum IL-6 and both probing depth and attachment loss where r=0.927 and 0.989 respectively.

Table 6 Correlation between serum cytokines and periodontal measurements

Periodontal measurements	Serum TNF-α		Serum IL-6	
	Pearson's r	p-value	Pearson's r	p-value
Plaque index	0.601	0.000	0.602	0.000
Gingival index	0.614	0.000	0.605	0.000
Probing depth (mm)	0.942	0.000	0.927	0.000
Attachment loss (mm)	0.995	0.000	0.989	0.000

DISCUSSION

Periodontal disease and diabetes are strongly interrelated and have common pathobiology. It is proposed that a two-way relationship is present between diabetes and periodontal

diseases. Most studies demonstrated that diabetes is a risk factor for gingivitis and periodontitis, and the glycemic control appears to be an important factor in this relationship[47-48]. On the other hand, periodontal diseases can have a significant impact on the metabolic state in diabetes. The presence of periodontitis increases the risk of worsening of glycemic control over time.

Our results showed that all glycemic parameters (HbAc1, FPG, HOMA-IR) were correlated with the level of glycemic control in DMT2 patients where the poorer the control the worsen were the glycemic readings. Periodontal measures including; plaque and gingival score, probing pocket depth and attachment losswere all significantly higher in the less-controlled diabetic subjects compared with well-controlled diabetics and healthy controls.

We verified the hypothesis that there is a strong positive correlation between serum and saliva TNF-α and IL-6. Also a very strong positive correlation was found between serum cytokines (TNF-α & IL-6) and all metabolic parameters (HbAc1, FPG, fasting insulin and HOMA-IR). Likewise, serum cytokines (TNF-α & IL-6) showed a strong positive correlation with both probing depth and attachment loss, and a moderate positive correlation with both PI and GI. Similarly, saliva cytokines (TNF-α & IL-6) have similar correlations with glycemic parameters, probing depth, attachment loss, in addition to plaque and gingival indices.

The positive correlation we found between periodontal indices and metabolic (glycemic) parameters was confirmed by many studies which stated that surgical and non-surgical periodontal treatment improved periodontal inflammatory conditions in DMT2 along with an improvement in metabolic control and lower HbA1c levels[49-50].It was suggested that this correlation may be due to locally produced cytokines which increased in periodontitis models and reach the systemic circulation[51-53]to promote IR in peripheral tissues[52-55].

The significant increase we documented in pocket depth and attachment loss in relation to glycemic control suggested that the risk for alveolar bone loss is greater, and bone loss progression more severe, for subjects with poorly controlled diabetes. This could be explained by the negative effect ofdiabeteson microvasculature beds, includingsoft tissues and alveolar bone supporting teeth resulting in prevalence of osteolytic lesions and bone loss **[**14]. Studies have provided evidence thatcontrol of periodontal infection has an impact on improvement of glycemic control evidenced by a decrease in the demand of insulin and decreased HBA1c levels[3].

The effect of glycemic control level on periodontal disease severity in DMT2 patients was also suggested to be due to theinteraction between bacteria products and PMN cells resulting in connective tissue destruction which occurs in patients with poorly-controlled diabetes and periodontal diseases[56-57]. Furthermore, it has been proposed that patients with poorly-controlled diabetes have more severe periodontal disease due to the increased production and accumulation of glucose mediated advanced glycation end products (AGEs) in the periodontal tissues as compared to patients with well-controlled diabetes which act as proinflammatory mediators, increase vascular permeability and increase binding cells, including macrophages, to induce secretion of a variety of cytokines[11,58].

Many clinical studies confirmed our finding that the levels of TNF-α and IL-6 in both serum and saliva of diabetics with periodontal disease were significantly higher than in systemically healthy subjects with periodontal disease[11,59-61]. This is said to be due to the effect of both cytokines on the periodontium in diabetic patients. TNF-α can stimulateproliferation, multiplication and differentiation of osteoclast pioneer cells and stimulate bone resorption by indirectly activating matured osteoclasts causing periodontal tissue destruction [10]. TNF-α is also released into the peripheral blood flow and provokes negative changesin immune responses challenges [7,12] activating up-regulation of inflammatory reactions and promoting mesenchymal cells to synthesize matrix metalloproteinproteases that in turn may be related to bone resorption and destruction of connectivetissue [32]. Likewise, IL-6 also has a similar effect on bone where it was found that osteoblasts secrete IL-6 to stimulate osteoclast formation as it is secreted by T cells and macrophages to stimulate immune response, during infection and after trauma, leading to inflammation[34]. This explains why in the presence of similar amounts of dental plaque and calculus, patients with DMT2 and periodontal disease had more severe periodontalproblems than healthy subjects with periodontal disease confirming that diabetes mellitus is a co-factor in the onset and evolution of periodontal disease.

Although some studies could find no correlation between serum IL-6 levels and serum TNF-α or glycaemia or HbAc1, yet a positive correlations with a tendency for statistical significance were found between salivary IL-6 levels in diabetics, FPGand HbAc1, and also between serum and salivary TNF-α levels [11].

Our results confirming the correlation of cytokines level with the severity of periodontal disease in DMT2 is suggested to be due to increased monocytic secretion of cytokines in diabetic patients which may be a consequence of a systemic response trait and that the presence of gram-negative infections such as periodontal diseases may interact synergistically to produce high local levels of these mediators and a more severe periodontal condition[62].

In addition, the cytokine induced inflammatory state in periodontitis can contribute to the overall low-grade inflammation that occurs in diabetes. This low-grade inflammation is characterized by chronic activation of the patient's innate immunity and, consequently, may aggravate IR and adversely affect glycemic control[63].

CONCLUSION

The clinical importance of our findings is that serum and saliva IL-6 & TNF-α detection might serve as an indicator to predict the evolution and severity of periodontal disease in subjects with DMT2. This will allow us to focus more on prevention and health promotion rather than diagnosis and treatment.

Recommendations

Joint efforts is crucial to design a chairside portable device for the detection of key salivary biomarkers associated with the inflammatory patterns and bone remodeling process through which a patient may undergofor saliva cytokines level detection, which will have significant impact on periodontal health promotion.

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