Whole Salivary Interleukin-6 and Matrix Metalloproteinase-8 Levels in Patients With Chronic Periodontitis With and Without Prediabetes

Fawad Javed,* Hameeda Bashir Ahmed,† Anwar Saeed,‡ Abid Mehmood,§ and Crawford Bain

Background: The cytokine profile in unstimulated whole saliva (UWS) of patients with prediabetes and chronic periodontitis (CP) remains uninvestigated. The aim of this study is to assess interleukin (IL)-6 and matrix metalloproteinase (MMP)-8 levels in UWS of patients with CP with and without prediabetes.

Methods: Eighty-eight males (aged 39 to 51 years) were divided into three groups: group 1: 28 patients with CP and prediabetes; group 2: 30 patients with CP and without prediabetes; and group 3: 30 controls. Fasting blood glucose (FBG) and hemoglobin A1c (HbA1c) levels, periodontal parameters (plaque index, bleeding on probing, probing depth, attachment loss, and marginal bone loss), and number of missing teeth were recorded. UWS samples were collected, and UWS flow rate (UWSFR) was measured. IL-6 and MMP-8 were measured in UWS using enzyme-linked immunosorbent assay. P values <0.05 were considered statistically significant.

Results: Mean FBG and HbA1c levels were significantly higher in group 1 (119.3 ± 3.1 mg/dL and 6.1% ± 0.2%) than group 2 (80.1 ± 3.5 mg/dL and 4.8% ± 0.5%; P <0.001) and group 3 (75.3 ± 2.2 mg/dL and 4.3% ± 0.2%; P <0.05). UWSFR was significantly higher in groups 2 (0.53 ± 0.1 mL/minute; P <0.05) and 3 (0.51 ± 0.1 mL/minute; P <0.01) than group 1 (0.33 ± 0.05 mL/minute). Periodontal parameters were worse in group 1 (P <0.05) and group 2 (P <0.05) than group 3. There was no difference in periodontal parameters, numbers of missing teeth, or salivary IL-6 and MMP-8 levels between patients in groups 1 and 2.

Conclusion: Salivary IL-6 and MMP-8 levels are elevated in patients with CP with and without prediabetes. J Periodontol 2014;85:e130-e135.

KEY WORDS
Chronic periodontitis; interleukins; matrix metalloproteinases; prediabetic state; saliva.

Recent studies1-3 have shown that chronic periodontitis (CP) is a common manifestation in patients with impaired glucose tolerance (or prediabetes) compared with systemically healthy controls. An explanation may be that persistent hyperglycemia in patients with prediabetes creates an imbalance between periodontopathogens and the host response, thereby causing overexpression of proinflammatory cytokines, formation of advanced glycation end products, dysfunction of polymorphonuclear leukocytes, and ultimately breakdown of supporting connective tissue attachment and alveolar bone.4,5

Conventionally, clinical and radiologic investigations (such as plaque index [PI], bleeding on probing [BOP], probing depth [PD], attachment loss [AL], and marginal bone loss [MBL]) are performed to assess and diagnose periodontitis in immunocompromised and systemically healthy individuals;3,6,7 however, laboratory-based investigations may also yield valuable information in this regard.8-12

Saliva is a complex oral fluid that can be easily collected using non-invasive methods. Studies9-11,13 have reported that various types of inflammatory biomarkers associated with oral and systemic diseases exist in saliva that make it a potential diagnostic fluid for oral inflammatory conditions such as CP.14,15
Inflammatory and immune mediators of periodontitis such as interleukin (IL)-6 and matrix metalloproteinase (MMP)-8 have been identified in saliva.\(^1\)\(^6\)\(^7\) IL-6 is an inflammatory mediator that promotes osteoclastic activity and bone resorption in patients with CP,\(^1\)\(^8\) whereas MMP-8 (also known as collagenase-2) is a major destructive metalloproteinase that plays an active role in the degradation and remodeling of the extracellular matrix.\(^1\)\(^9\) Because CP is a common activity and bone resorption in patients with CP, it is hypothesized that unstimulated whole saliva (UWS) concentrations of IL-6 and MMP-8 are elevated in patients with CP and prediabetes compared with otherwise medically healthy individuals with CP and controls (medically healthy individuals without periodontitis). To the authors’ knowledge from indexed literature, this hypothesis has not been tested before.

The aim of the present study is to assess IL-6 and MMP-8 levels in UWS of patients with CP with and without prediabetes.

**MATERIALS AND METHODS**

**Ethical Guidelines**

The study protocol was reviewed and approved by the research ethics review committee of Jinnah Postgraduate Medical Center (JPMC), Karachi, Pakistan. Consentig individuals were asked to read and sign a consent form. It was mandatory for consenting individuals to have read and signed the consent form before being included in the present investigation.

**Inclusion and Exclusion Criteria**

Individuals with medically diagnosed prediabetes (fasting blood glucose [FBG] 100 to 125 mg/dL [5.6 to 6.9 mmol/L] and hemoglobin A1c [HbA1c] 5.7% to 6.4%)\(^2\)\(^0\) were included. Exclusion criteria encompassed the following: 1) self-reported systemic diseases including type 1 and type 2 diabetes mellitus, human immunodeficiency virus infection/acquired immune deficiency syndrome, cardiovascular disorders, epilepsy, hepatic disorders, and renal disorders; 2) antibiotic and/or steroid intake within the past 3 months; 3) misaligned teeth; 4) edentulism; 5) self-reported habitual tobacco use (smoking and/or chewing) and alcohol consumption; 6) history of periodontal treatment within 6 months; and 7) pregnancy.

**Recruitment of Study Participants and Grouping**

The present cross-sectional study was conducted from February 2013 to June 2013 in which 88 male participants (aged 39 to 51 years) volunteered to participate. Group 1 consisted of 30 patients with medically diagnosed prediabetes who were recruited from the diabetes care unit of JPMC, Karachi, Pakistan. In group 2, 30 self-reported systemically healthy patients with CP were recruited from the Department of Dentistry, JPMC. Group 3 (control) included 28 self-reported systemically healthy individuals without CP who were recruited from a residential area and invited to the Department of Dentistry, Jinnah Hospital, for periodontal assessment and saliva collection. Individuals with prediabetes were requested to present their medical records to confirm the diagnosis of prediabetes.

All participants were invited to an oral health care center at early morning hours for UWS collection and periodontal examination. All participants were asked to visit the oral health care center in a fasting state.

**Collection of UWS Samples**

The UWS samples were collected as described elsewhere.\(^2\)\(^1\) In summary, for UWS collection, all individuals (N = 88) were comfortably seated on a chair and requested to expectorate (without swallowing) into a gauged measuring cylinder for 5 continuous minutes. UWS flow rate (UWSFR) was measured and recorded in milliliters per minute. Immediately after collection, UWS samples were placed on ice and aliquoted before freezing at −80°C. UWS samples were analyzed within 6 months of collection.

**Measurement of IL-6 and MMP-8 Levels in UWS**

Concentrations of IL-6 and MMP-8 in UWS samples were determined in duplicate from each individual using enzyme-linked immunosorbent assay. Human IL-6 kits\(^\#\) and MMP-8 kits\(^\&\) were used according to the manufacturers’ instructions. In summary, a standard curve was constructed using standards provided with the IL-6 and MMP-8 kits, and protein concentrations were calculated from the standard curve. A total of 100 µL diluted standards with samples was dispensed, in duplicate, into the wells coated with a specific protein antibody. The plates were incubated at room temperature for 60 minutes, after which they were washed three times with a wash solution. Conjugate solution (100 µL) was added, and the plates were incubated at room temperature for another 120 minutes. The wells were washed once again three times with a wash solution, and 100 µL substrate solution was added. The plates were incubated for 20 minutes at room temperature, after which 50 µL stop solution was added to terminate color development. Absorbance was determined by reading the plate at 450 nm in a spectrophotometer.**

**Measurement of FBG and HbA1c Levels**

In all 88 participants, FBG levels were measured using a digital glucometer\(^\dagger\)\(^\dagger\) and expressed in mg/dL; HbA1c levels were measured by ion-exchange high-

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\(^1\) Human IL-6 Quantikine ELISA Kit, R&D Systems, Minneapolis, MN.

\(^\#\) Human MMP-8 Quantikine ELISA Kit, R&D Systems.

\(^\&\) EL800 Universal Microplate Reader, BIO-TEK Instruments, Winooski, VT.

\(^\dagger\) Accu-Chek Activ, Roche Diagnostics, Mannheim, Germany.
performance liquid chromatography and expressed as percentages.

Clinical Periodontal Examination
Clinical periodontal examination was performed by a trained and calibrated investigator (AM) who was masked to the groups. The overall $k$ for intra-examiner reliability was 0.78. Full-mouth PI, BOP, PD, and AL were measured at six sites per tooth (mesio-buccal, mid-buccal, disto-buccal, disto-lingual/palatal, mid-lingual/palatal, and mesio-lingual/palatal) on all maxillary and mandibular teeth (excluding third molars). PD was measured to the nearest millimeter with a graded probe.‡‡ Fractured teeth with embedded root remnants were not assessed.

Panoramic radiographs were taken using a digital panoramic tomography machine§§ and viewed on a calibrated computer screen using a software program.¶¶ MBL was gauged as the vertical distance from 2 mm below the cemento-enamel junction (CEJ) to the most apical part of marginal bone. HbA1c levels were significantly higher among participants in group 1 compared with those in groups 2 ($P < 0.01$) and 3 ($P < 0.01$). A family history of diabetes was more often reported by individuals in groups 1 and 2 compared with those in group 3 ($P < 0.01$). All patients in group 1 had been recommended by their health care providers to follow dietary control regimens for management of their prediabetic state (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group 1 (n = 30)</th>
<th>Group 2 (n = 30)</th>
<th>Group 3 (n = 28)</th>
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</thead>
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<tr>
<td>Male sex (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Mean ± SD age (years)</td>
<td>41.5 ± 2.5</td>
<td>42.2 ± 1.8</td>
<td>42.7 ± 3.2</td>
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<tr>
<td>Mean ± SD duration of prediabetes (months)</td>
<td>13.5 ± 1.4</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Mean ± SD FBG (mg/dL)</td>
<td>119.3 ± 3.1†</td>
<td>80.1 ± 3.5*</td>
<td>75.3 ± 2.2†</td>
</tr>
<tr>
<td>Mean ± SD HbA1c (%)</td>
<td>6.1 ± 0.2‡</td>
<td>4.8 ± 0.5‡</td>
<td>4.3 ± 0.2‡</td>
</tr>
<tr>
<td>Mean ± SD family history of diabetes (%)</td>
<td>42.5 ± 0.6§</td>
<td>30.4 ± 2.3§</td>
<td>8.5 ± 1.6§</td>
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<td>Treatment for prediabetes (%)</td>
<td> </td>
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<tr>
<td>Allopathic</td>
<td>—</td>
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<tr>
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</tr>
<tr>
<td>Other</td>
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</table>

* $P < 0.001$. Group 1 compared to Group 2.
† $P < 0.05$. Group 1 compared to Group 3.
‡ $P < 0.01$. Group 1 compared to Group 2 and Group 3.
§ $P < 0.01$. Group 1 compared to Group 3 and Group 2 compared to Group 3.

Results
Characteristics of the Study Cohort
All participants (N = 88) were males. There was no significant difference in age among individuals in groups 1, 2, and 3. The mean duration of prediabetes among patients in group 1 was 13.5 ± 1.4 months. The mean FBG was significantly higher among participants in group 1 compared with those in groups 2 ($P < 0.001$) and 3 ($P < 0.05$). A family history of diabetes was more often reported by individuals in groups 1 and 2 compared with those in group 3 ($P < 0.01$). All patients in group 1 had been recommended by their health care providers to follow dietary control regimens for management of their prediabetic state (Table 1).

Periodontal Inflammatory Parameters
Periodontal inflammatory conditions (PI [$P < 0.01$], BOP [$P < 0.01$], PD > 4 mm and < 6 mm [$P < 0.01$], and AL [$P < 0.01$]) were worse among individuals in group 1 compared with controls (group 3). Scores of salivary cytokine concentrations were assessed using one-way analysis of variance. For multiple comparisons, Bonferroni post hoc adjustment test was used. $P$ values < 0.05 were considered statistically significant.
PI (P<0.01), BOP (P<0.01), PD >4 mm and <6 mm (P<0.01), and AL (P<0.01) were significantly higher among individuals in group 2 than those in group 3 (Table 2). MBL was significantly higher among patients in groups 1 (P<0.01) and 2 (P<0.01) compared with those in group 3.

Numbers of missing teeth were significantly higher among individuals in groups 1 (P<0.01) and 2 (P<0.01) compared with group 3 (1.2–0.2). There was no difference in scores of periodontal parameters and numbers of missing teeth among patients in groups 1 and 2 (Table 2).

**UWSFR**
The UWSFR was significantly higher among individuals in groups 2 (0.53 ± 0.1 mL/minute) (P<0.05) and 3 (0.51 ± 0.1 mL/minute) (P<0.01) compared with patients in group 1 (0.33 ± 0.05 mL/minute).

**Salivary IL-6 and MMP-8 Levels**
Salivary IL-6 levels were significantly higher among patients in groups 1 (P<0.01) and 2 (P<0.01) compared with individuals in group 3. There was no significant difference in salivary IL-6 levels among individuals in groups 1 and 2 (Fig. 1A).

Salivary MMP-8 levels were significantly higher among patients in groups 1 (P<0.01) and 2 (P<0.05) compared with individuals in group 3. There was no significant difference in salivary MMP-8 levels among individuals in groups 1 and 2 (Fig. 1B).

**DISCUSSION**
In the present study, it is hypothesized that levels of salivary IL-6 and MMP-8 are significantly higher in patients with CP and prediabetes (group 1) compared with individuals with CP without prediabetes (group 2) and controls (group 3). Because chronic hyperglycemia systemically promotes a proinflammatory state chiefly owing to interactions between advanced glycation end products and their receptors, it is suggested that there are two chronic inflammatory conditions in prediabetic patients with periodontal disease, each of which affects the other.

The present results demonstrated significantly higher salivary IL-6 and MMP-8 levels among patients in group 1 (patients with prediabetes and CP) and group 2 (patients with CP without prediabetes) compared with individuals in group 3 (controls). Interestingly, no significant difference was found in salivary IL-6 and MMP-8 levels among patients in group 1 compared with group 2. Various explanations may be posed in this regard. First, it has been reported that severity of periodontal disease in patients with diabetes is associated with the duration of the chronic hyperglycemic state. In the present study, duration of prediabetes among patients in group 1 was approximately 12 months. It is therefore speculated that duration of prediabetes among participants of the present study was not long enough to augment the periodontal inflammatory state previously existing in these patients. Patients with CP with a longstanding history of prediabetes may experience more severe CP than patients with CP with a short medical history of prediabetes. This may in turn influence the salivary cytokine levels in these patients. Further studies are warranted to test this hypothesis.

Conventionally, fasting and random blood glucose levels are used to gauge glycemic levels in individuals with and without hyperglycemia; however, the aforementioned techniques are unable to gauge metabolic control in the long term. It has been
suggested that HbA1c measurement is a valuable tool for measuring glycemic control, as it provides a good estimation of the average blood glucose level over the previous 30- to 90-day period.29 HbA1c was therefore used as an adjunct to FBG measurement in the present study.

It is noteworthy that all individuals participating in the present study were males. Studies30,31 have reported that multiple episodes of pregnancy, recurrent gestational diabetes mellitus, and obesity are significant risk factors of prediabetes among females. The authors hypothesize that the periodontal status is worse in prediabetic females compared with males with prediabetes. It is therefore tempting to speculate that proinflammatory cytokine levels in oral fluids (such as UWS and gingival crevicular fluid [GCF]) may also vary among males and females with prediabetes. However, further studies are warranted to assess the effect of habitual tobacco product usage on GCF and salivary cytokine profiles among prediabetic smokers and tobacco chewers compared with prediabetic individuals not using any form of tobacco.

CONCLUSION
Within the limits of the present study, it is concluded that salivary IL-6 and MMP-8 levels are elevated in patients with CP with and without prediabetes.

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