

## Periodontal parameters in prediabetes, type 2 diabetes mellitus, and non-diabetic patients

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**Abstract:** The aim of the present study was to compare the clinical and radiographic periodontal parameters in prediabetes, type 2 diabetes mellitus (T2DM), and non-diabetic patients. Forty-one patients with prediabetes (Group 1), 43 patients with T2DM (Group 2), and 41 controls (Group 3) were included. Demographic data were recorded using a questionnaire. Full-mouth clinical (plaque index [PI], bleeding on probing [BOP], probing depth [PD], clinical attachment loss [CAL], missing teeth [MT]) and radiographic (marginal bone loss [MBL]) parameters were measured on digital radiographs. In all groups, hemoglobin A1c (HbA1c) levels were also measured. P values less than 0.05 were considered statistically significant. The mean age and HbA1c levels of participants in Groups 1, 2, and 3 were  $53.4 \pm 3.5$ ,  $60.1 \pm 0.6$ , and  $56.6 \pm 2.5$  years and 6.1%, 8.4%, and 4.8%, respectively. The mean duration of prediabetes and T2DM in patients from Groups 1 and 2 were  $1.9 \pm 0.3$  and  $3.1 \pm 0.5$  years, respectively. PI, BOP, PD, MT, CAL, and MBL were significantly higher in Groups 1 ( $p < 0.05$ ) and 2 ( $p < 0.05$ ) than in Group 3. There was no statistically significant difference in these parameters in Groups 1 and 2. Periodontal parameters were worse between prediabetes and T2DM patients compared with controls; however, these parameters were comparable between prediabetes and T2DM patients.

**Keywords:** Alveolar Bone Loss; Diabetes Mellitus, Type 2; Periodontal Index; Prediabetic State; Periodontitis.

### Introduction

It is well known that periodontal inflammation is significantly higher in patients with chronic hyperglycemia (such as patients with prediabetes and with poorly-controlled type 2 diabetes mellitus [T2DM]) than in healthy controls.<sup>1,2</sup> One explanation for these results is that chronic hyperglycemia increases the expression of toll-like receptors (TLRs) and proinflammatory cytokines (such as interleukin [IL] 1-beta and tumor necrosis factor alpha) in periodontal tissues, thereby augmenting periodontal inflammation.<sup>3</sup> Moreover, chronic hyperglycemia has also been reported to induce a state of oxidative stress that disrupts the equilibrium between the production and the inactivation of reactive oxygen species (ROS).<sup>4</sup> In addition, persistent hyperglycemia has been associated with increased formation and



accumulation of advanced glycation end products (AGEs) in periodontal tissues, which in turn make periodontal inflammation worse.<sup>5</sup>

Recently, Costa et al.<sup>1</sup> investigated the influence of increased hemoglobin A1c (HbA1c) levels on the progression of periodontal disease in patients with chronic hyperglycemia. The results showed that the progression of periodontal disease is associated with an increase in HbA1c levels in hyperglycemic patients.<sup>1</sup> Evidence from clinical studies have also shown that maintenance of blood glucose levels (via strategies such as dietary control and/or antihyperglycemic medication) significantly reduces the severity of clinical (such as plaque index [PI], bleeding on probing [BOP], probing depth [PD], clinical attachment loss [CAL]) and radiographic (marginal bone loss [MBL]) periodontal parameters between prediabetes and T2DM patients.<sup>6,7,8</sup> However, it is pertinent to mention that these studies primarily compared their respective non-diabetic controls with patients either with prediabetes or T2DM. To our knowledge from the indexed literature, only one study has compared periodontal parameters in prediabetes and T2DM patients.<sup>9</sup> Interestingly, the results of that study were contradictory to earlier reports as prediabetes patients and controls had comparable periodontal statuses. These results should be interpreted with caution due to the fact that all participants in the study by Altamash et al.<sup>9</sup> were overweight (a significant risk factor for periodontal inflammation, which could have biased the results) and nearly 50% of the controls were unaware of their hyperglycemic state. Hence, comparison of periodontal inflammatory status in prediabetes and T2DM patients warrants further well-designed and well-controlled investigations.

In the present study, it is hypothesized that (a) periodontal parameters are worse in prediabetes and T2DM patients than in controls; (b) periodontal parameters are worse in T2DM patients than in prediabetes patients since hyperglycemic levels are higher in the former group of individuals. Therefore, the aim of the present cross-sectional cohort study was to compare the clinical and radiographic periodontal parameters in prediabetes and T2DM patients and healthy controls.

## Methods

### Ethical guidelines

The study was performed in accordance with the Declaration of Helsinki. An information sheet printed in simple English and Arabic describing the purpose and methods used in the present study was provided to individuals. The information sheet also clearly stated that participation was completely voluntary and the individuals reserved the right to withdraw from the study at any time without any consequences.

### Inclusion and exclusion criteria

The inclusion criteria were as follows: (a) individuals with medically diagnosed prediabetes (HbA1c levels between 5.7% and 6.4%);<sup>10</sup> (b) individuals with medically diagnosed T2DM (HbA1c levels  $\geq$  6.5%),<sup>10</sup> and (c) non-diabetic controls (HbA1c levels 4% to 5%).<sup>10</sup> Exclusion criteria were (a) patients with self-reported systemic diseases such as acquired immune deficiency syndrome, cardiovascular disorders, hepatic disorders, renal disorders, and obese/overweight individuals (body mass index according to Asian classification:  $\geq$ 27.5 kg/m<sup>2</sup>);<sup>11</sup> (b) use of antibiotics and/or steroids within the past 90 days; (c) patients who had undergone periodontal therapy within the past 90 days; (d) patients with crowding of teeth or occlusal trauma; (e) edentulous individuals; (f) habitual alcohol and tobacco use; (g) pregnancy and/or lactation; and (h) maxillary and mandibular third molar.

### Study participants and groups

The study was conducted from December 2015 to June 2016. Subjects were categorized into three groups based on HbA1c levels. The three groups were: (1) Group 1 – prediabetes patients, (2) Group 2 – T2DM patients, and (3) Group 3 – healthy controls. All participants who self-reported to have prediabetes or T2DM were requested to present their medical records for verification of HbA1c levels including the diagnosis of prediabetes and of diabetes mellitus.

### Questionnaire

All participants completed a baseline questionnaire provided by a trained interviewer (FV) that included data regarding (1) age; (2) sex; (3) duration of prediabetes

or T2DM; (4) family history of prediabetes or T2DM; (5) treatment of prediabetes or T2DM recommended by healthcare provider(s) and; (6) daily oral home care including toothbrushing and flossing.

### Clinical periodontal parameters

One periodontal examiner (ZA) who was blinded to the study groups performed the periodontal assessment. The overall *k* value for intraexaminer reliability was 0.9. Clinical parameters, including PI, BOP, PD, and clinical AL for all teeth, were measured at six sites (mesiobuccal, mid-buccal, distobuccal, distolingual/palatal, mid-lingual/palatal, and mesiolingual/palatal) on all maxillary and mandibular teeth.<sup>12,13,14</sup> Probing depths were assessed using a metallic color-coded UNC-15 probe (Hu-Friedy, Chicago, USA).

### Marginal bone loss

Digital bitewing radiographs (Ektaspeed plus; Kodak, Rochester, NY, USA) were taken of all teeth and displayed on a calibrated computer screen (Samsung SyncMaster digital TV monitor, Suwon City, Gyeonggi-do, Korea) and were analyzed by a software program (Image Tool 3.0, Department of Dental Diagnostic Science, University of Texas Health Science Center, San Antonio, TX, USA). Marginal bone loss, i.e. the lost bone from 2 mm below the cemento-enamel junction (CEJ) to the most coronal part of alveolar bone, was evaluated on all present teeth.<sup>8</sup> Local factors such as cavitated teeth, restored teeth, misaligned teeth, and/or poor X-ray quality that obscured the visibility of the bone crest were excluded. All X-rays were assessed by one trained examiner (FV). The overall *kappa* score for intraexaminer reliability was 0.92.

### Assessment of hemoglobin A1c levels

Glycemic status was assessed through medical records and a new HbA1c test was performed to assess glycated hemoglobin levels during the last 3 months. Non-diabetic status was also confirmed by an HbA1c test. The groups were classified according to the American Diabetes Association criteria.<sup>10</sup> In all groups, chair-side HbA1c levels were measured using an HbA1c analyzer kit (Quo-Test, EKF Diagnostics,

Magdeburg, Germany). Blood samples were drawn from the patients and controls between 8.00 a.m. and 10.00 a.m. by venipuncture of an antecubital vein. Blood samples were collected in Vacutainer tubes with a separation gel and in heparinized tubes for HbA1c measurements and were centrifuged at 2,000 rpm for 15 min at 4 °C after an incubation period of 30 min.

### Statistical analyses

Statistical analysis was performed using a software program (SPSS v.18, IBM, Chicago, IL., USA). Clinical and radiographic periodontal parameters in prediabetes and T2DM patients and controls were assessed using the Kruskal-Wallis test. Multiple logistic regression analysis was also performed to investigate the associations between periodontal inflammation in prediabetes and T2DM patients and controls after adjustment of the data for toothbrushing habits. Bonferroni *post-hoc* adjustment was used for multiple comparisons. Multivariate regression analysis was employed to identify explanatory variables for periodontal outcomes, controlling for the effect of possible covariates such as HbA1c levels and oral hygiene care. The direction and strength of association between MBL and covariates were assessed by generating odds ratios, the precision of which could be measured by 95% confidence intervals. Power and sample sizes were calculated using a computer software tool (nQuery Advisor 6.0, Statistical Solutions, Saugas, MA., USA). With the inclusion of 40 individuals per group (assuming a standard deviation of 1.0% for CAL, which is achieved by adding the values of PD and gingival recession), the study power was estimated to be 90% with a two-sided significance level of 0.05. P values < 0.05 were considered statistically significant.

## Results

### General characteristics of the study cohort

In total, 41 patients in Group 1, 43 in Group 2, and 41 in Group 3 volunteered to participate in the present study. The mean age of participants in Groups 1, 2, and 3 were 53.4, 60.1, and 56.6 years, respectively. The mean HbA1c levels of participants in Groups 1, 2, and 3 were 6.1%, 8.4%, and 4.6%, respectively. The mean

duration of prediabetes and T2DM in patients from Groups 1 and 2 were 1.9 and 3.1 years, respectively. A family history of diabetes was reported by 28 individuals in Group 1 and 31 individuals in Group 2 compared with controls (n = 7). Three, five, and 26 individuals in Groups 1, 2, and 3, respectively, reported having college education. All individuals in Group 1 (n = 41) were advised by their healthcare providers to maintain their blood glucose levels via dietary controls. In Group 2, all participants were prescribed antihyperglycemic medications for the treatment of T2DM and were also advised to observe dietary control. Toothbrushing once daily was reported by 87.8%, 81.4%, and 70.7% of individuals in Groups 1, 2, and 3, respectively (Table 1). None of the participants reported flossing their teeth.

### Periodontal parameters in participants from Groups 1, 2, and 3

Periodontal parameters and the extent of disease severity in all groups are shown in Table 2. PI, BOP, PD, CAL, MBL, and the number of MT were statistically significantly higher in patients from Groups 1 (p < 0.05) and 2 (p < 0.05) compared with Group 3. There was no statistically significant difference in PI, BOP, PD, CAL, MBL, and in the number of MT in participants from Groups 1 and 2 (Table 2).

There was no statistically significant difference in PI, BOP, PD, CAL, MBL, and in the number of MT

in individuals who reported brushing their teeth once daily compared with those in Groups 1, 2, and 3, who brushed twice daily.

### Regression analysis to control HbA1c levels and oral hygiene

As MBL was considered a strong indicator of periodontal inflammation, HbA1c levels and brushing frequency were adjusted taking only MBL into consideration. The multivariate regression analysis revealed that MBL showed a statistically significant difference between prediabetic and T2DM patients even after adjusting for HbA1c levels and brushing frequency in both groups (p < 0.05).

### Discussion

The present study was based on two hypotheses: periodontal parameters are worse in prediabetes and T2DM patients than in controls; and periodontal parameters are worse in T2DM patients than in prediabetes patients since hyperglycemic levels are higher in the former group of individuals. The present results support the first hypothesis and several studies have shown that periodontal parameters are worse in hyperglycemic than in normoglycemic individuals.<sup>6,8,15</sup> One explanation for this is that chronic hyperglycemia increases the production of proinflammatory cytokines such as

**Table 1.** General characteristics of the study population.

Parameters	Group 1	Group 2	Group 3
	(Patients with prediabetes)	(Patients with T2DM)	(Controls)
Number of participants	41	43	41
Mean age (±SD) in years	53.4 ± 3.5	60.1 ± 0.6	56.6 ± 2.5
Mean duration (±SD) of the endocrine disorder in years	1.9 ± 0.3	3.1 ± 0.5	—
Mean hemoglobin A1c levels (range)	6.1 (5.8–6.3)	8.4 (8.1–9.3)	4.5 (4.3–4.8)
Family history of diabetes	28	31	7
Schooling (%)			
Secondary education	92.7	88.4	36.6
College education	7.3	11.6	63.4
Treatment of hyperglycemia			
Dietary control	41	0	—
Antihyperglycemic medications + dietary control	0	43	—
Toothbrushing			
Once daily	36	35	29
Twice daily	5	8	12

**Table 2.** Scores of periodontal parameters in participants from Groups 1, 2, and 3.

Parameters	Group 1	Group 2	Group 3
	(Patients with prediabetes)	(Patients with T2DM)	(Controls)
Number of participants	41	43	41
Plaque index (%) (range)	46.5 (41.5–54.1)*	49.2 (44.6–53.7)*	20.4 (17.1–25.7)
Bleeding on probing (%) (range)	50.6 (45.2–60.3)*	55.3 (51.6–66.4)*	23.5 (17.5–28.4)
Probing depth in mm (range)	5.2 (4.5–5.8)*	5.8 (4.8–6.4)*	2.5 (1–3)
Sites ≤ 3 mm probing depth (%)	57.3 ± 21.7	35.8 ± 11.6	88.5 ± 21.7
Sites 4–6 mm probing depth (%)	38.5 ± 9.7	51.3 ± 22.3	5.7 ± 1.8
Sites ≥ 7 mm probing depth (%)	4.2 ± 1.9	12.9 ± 6.6	5.8 ± 3.1
Clinical attachment loss in mm (range)	3.5 (2.4–4)*	3.8 (2.2–4.6)*	0.6 (0–1.2)
Sites ≤ 3 mm attachment loss (%)	71.6 ± 38.7	29.7 ± 11.6	92.3 ± 21.7
Sites 4–6 mm attachment loss (%)	21.5 ± 14.7	61.3 ± 21.7	6.5 ± 2.4
Sites ≥ 7 mm attachment loss (%)	6.9 ± 3.8	9.0 ± 3.4	1.2 ± 0.2
Marginal bone loss in mm (range)	4.2 (3.8–5.3)*	4.7 (3.6–5.5)*	2.2 (1.5–2.5)
Number of missing teeth (range)	10.4 (5–14)*	13.2 (8–15)*	4.8 (0–9)

\*Compared with Group 3 ( $p < 0.05$ ).

IL-6 by human gingival fibroblasts as compared to normal glucose.<sup>4</sup> Moreover, results obtained from experimental studies indicate that the interaction between AGEs and their receptors is significantly higher in the inflamed periodontal tissues of rats with induced hyperglycemia than in normoglycemic rats.<sup>16,17</sup> Furthermore, Promsudthi et al.<sup>18</sup> reported that hyperglycemia increases the expression of TLRs (which contribute to a greater inflammatory response in hyperglycemic patients and to periodontal disease) in periodontal tissues. This suggests that as the severity of hyperglycemia increases, periodontal inflammatory response is also expected to rise. We therefore speculated that periodontal parameters are worse in T2DM patients than in prediabetic individuals (second hypothesis). However, the present study showed no statistically significant difference in PI, BOP, PD, CAL, MBL, and in the number of MT when prediabetic patients were compared with T2DM patients. Various explanations can be given for that.

There have been reports of a statistically significant relationship between duration of hyperglycemia and the severity of periodontal inflammation.<sup>19,20</sup> According to Al-Shammari et al.,<sup>6</sup> periodontal parameters (MT and CAL) are significantly higher in patients with a longer duration of T2DM ( $\geq 5$  years) compared with individuals with a shorter duration of diabetes ( $< 5$  years). In the present study, the duration of prediabetes and T2DM in Group 1 and Group 2 patients was approximately 2 years and 3 years, respectively. The duration of prediabetic/

diabetic status was short, thus limiting the effect of hyperglycemia. This could have restricted the progress of periodontal disease resulting in comparable outcomes between the two groups in the present study. Another factor that may be associated with similar periodontal parameters in individuals from Groups 1 and 2 is the interaction between AGEs and their receptors in chronic hyperglycemic state. In chronic hyperglycemia, numerous proteins undergo nonenzymatic glycosylation, which leads to excessive formation of AGEs, making the tissues more susceptible to deterioration by altering the collagen structure and making it less soluble for normal repair.<sup>20</sup> Furthermore, AGEs are linked to the enhanced production of proinflammatory cytokines such as interleukins and matrix metalloproteinases.<sup>21</sup> In addition, chronic hyperglycemia alters host tissues and physiology, which may weaken the hosts' barrier function and immune defense against periodontal pathogens by impairing the chemotactic and phagocytic function of neutrophils, predisposing to destructive peri-implant damage.<sup>22</sup> The severity of inflammation induced by interactions between AGEs and their receptors was possibly comparable because of the relatively short history of hyperglycemia in patients from Groups 1 and 2. Nonetheless, the present results suggest that assessment of HbA1c levels is a valuable means to explain signs of periodontal inflammatory conditions in patients with newly diagnosed prediabetes and T2DM. In addition, as the study was not powered to detect the differences between prediabetes and T2DM,

this may also have contributed towards the comparable outcomes between the two groups.

It is well known that poor schooling and family history of diabetes are significant risk factors for prediabetes, T2DM, and periodontal disease.<sup>8,23,24,25</sup> The present study supports these findings since a family history of diabetes was reported by approximately 68% and 72% of the patients in Groups 1 and 2, respectively, compared with individuals in Group 3 (~17%). Moreover, college education was more often reported by controls (~63%) compared with patients in Groups 1 (~7%) and 2 (~11%). It is therefore essential to educate patients (particularly those with a family history of diabetes) about the possible risk factors for prediabetes and diabetes and their influence on overall health. Routine community-based health awareness programs can play a role in this situation. An interesting finding in the present study was that even after stratifying the data on toothbrushing habits, periodontal parameters remained comparable in patients from Groups 1 and 2 compared with controls. One explanation is that there were only a limited number of patients in Groups 1 (~12%) and 2 (~19%) who reported brushing their teeth twice daily. Moreover, since schooling was poorer in patients from Groups 1 and 2 compared with controls, it is possible that their perception of oral hygiene maintenance also varied compared with controls.

A limitation of the present study was that strict eligibility criteria were imposed for patient selection. It is well known that habits such as tobacco smoking and smokeless tobacco consumption are risk factors

for periodontal disease.<sup>26,27</sup> Moreover, since most of the participants in the present study were male, it is exigent to assess whether there is a difference in the severity of periodontal disease between males and females with prediabetes and T2DM. Further studies are warranted in this regard. The data on the duration of the disease were retrospectively collected. The self-reported duration of glycemic status in prediabetes and T2DM patients relied on the patients' own recall abilities and, therefore, this may have affected the results of the present study. Also, the lack of accurate information on the time of diabetes onset is still a possible limitation. The cross-sectional nature of the data and the single analysis of HbA1c levels at baseline are other important limitations that should not be overlooked. In addition, it is well documented that obesity is associated with compromised periodontal parameters and even with poor periodontal therapeutic response, which may have biased the results.<sup>28,29</sup>

## Conclusion

Periodontal parameters were worse in prediabetes and T2DM patients than in controls; however, these parameters were comparable between prediabetes and T2DM patients.

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