Original Article

Salivary and crevicular fluid proinflammatory cytokines and advanced glycation end products in patients with different glycemic levels undergoing fixed orthodontic treatment

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ABSTRACT

Objective: To examine whether patients with different blood glycemic levels undergoing fixed orthodontic therapy demonstrate changes in the biochemical profiles of crevicular fluid and salivary advanced glycation end products (AGEs) and proinflammatory cytokine levels in comparison with nondiabetic healthy subjects.

Materials and Methods: Prediabetic subjects, subjects with type 2 diabetes mellitus (T2DM), and subjects without a diabetes mellitus diagnosis undergoing fixed orthodontic therapy with MBT prescription brackets (0.022-inch brackets and 0.019 × 0.025-inch stainless steel archwires) were included in the study. The following clinical periodontal parameters were evaluated: (1) plaque score (PS), (2) probing depth (PD), (3) bleeding on probing (BOP), and (4) clinical attachment loss. Crevicular fluid and saliva specimens were collected during regular orthodontic visits. Salivary and crevicular fluid tumor necrosis factor alpha, interleukin-6, ghrelin, resistin, AGEs, and receptor activator of nuclear factor κB ligand were evaluated using a human magnetic Luminex multiplex assay.

Results: BOP scores were significantly higher among T2DM subjects (19.2%) than among nondiabetic subjects (11.2%) and prediabetic subjects (15.9%). Comparable values were demonstrated by all three study groups regarding PD scores and PSs. T2DM subjects demonstrated higher scores for gingival crevicular fluid (GCF) chemokines than nondiabetic and prediabetic subjects. A statistically significant difference was found in the levels of AGEs and resistin among the three study groups. The scores revealed for the levels of GCF resistin and AGEs versus periodontal BOP demonstrated a significant positive association by the Pearson correlation test.

Conclusions: T2DM patients demonstrated significantly higher levels of GCF resistin and AGEs during fixed orthodontic therapy. Chronic hyperglycemic patients undergoing orthodontic therapy demonstrated a proinflammatory response. (*Angle Orthod*. 2024;94:233–239.)

KEY WORDS: Diabetes mellitus; Prediabetes; Chemokines; Crevicular fluid; Saliva; Orthodontic tooth movement

INTRODUCTION

Type 2 diabetes mellitus (T2DM), a chronic metabolic condition, has become a leading community health issue, and its prevalence has approximately quadrupled over the previous 4 decades.[1,2] T2DM is generally characterized by chronic hyperglycemia, which manifests as persistently high levels of blood glucose owing to either a decreased sensitivity to the normal impact of insulin or a slow alleviation of insulin generation by the body.[3,4] Published evidence suggests that the pathophysiology of T2DM is associated with a subclinical long-standing inflammatory condition. Chronic hyperglycemia is characterized by numerous proteins undergoing glycosylation leading to the generation of advanced glycation end products (AGEs) in the oral tissues.[5,6] The cross-linking of collagen fibers by AGEs together with their receptors lessens tissue solubility, resulting in decreased

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reparative efficacy of the periodontal hard and soft tissues.[7] Prediabetes is a state manifesting as increased glycemic parameters falling between the optimum levels and the diabetes threshold. Even though the particular diagnostic criteria for prediabetes might differ among various professional organizations globally, it is universally established as a high-risk condition for the inception of diabetes, with a per-annum conversion rate of 5% to 10%.[3] Research indicates that the intensity of inflammation in prediabetes may not be equal to that in T2DM; however, oxidative stress and the proinflammatory burden could be strong indicators of the progression of prediabetes to T2DM.[8]

Orthodontic tooth movement (OTM) involves the harmonious remodeling of periodontal soft tissues as a reaction to external force, leading to a localized aseptic inflammatory response.[9] This causes osteoclastic activity in regions of compression, whereas the deposition of osteoblasts occurs in regions of tension.[10,11] Several biomarkers are released during OTM, and most of these can be found in the gingival crevicular fluid (GCF). Among these biomarkers are tumor necrosis factor, interleukins (ILs), matrix metalloproteinases 8 and 9, and receptor activator of nuclear factor κB ligand.[6,12] Since evidence confirms an association between chronic systemic inflammation and increased glycated hemoglobin A1c (HbA1c) concentrations, the potential risks for periodontal disease in T2DM patients are well documented, however, there is little evidence that demonstrates OTM in chronic hyperglycemic patients in humans.[13]

In vivo investigations performed on rat models have assessed the effect of chronic hyperglycemia on OTM, with conflicting outcomes. Arita and colleagues[14] demonstrated an increase in the OTM rate, whereas Braga and colleagues[15] proposed that OTM was decreased. A recently conducted cross-sectional report on diabetic and healthy participants estimated the levels of proinflammatory cytokines in subjects undergoing OTM.[16] Nonetheless, no clinical studies or cross-sectional cohort reports have been undertaken in humans to assess the salivary and GCF levels of proinflammatory cytokines in populations undergoing OTM who have different hyperglycemic levels. The hypothesis explaining the impact of hyperglycemia on OTM involves alterations in biochemical molecules and proinflammatory cytokines that influence the rate of osteoclastic and osteoblastic activity. Likewise, investigations have assessed the influence of obesity on OTM and found that the rate of OTM might be significantly affected by proinflammatory alterations in the structure of the periodontium among obese people.[17] The aim of the current study was to examine whether people with different blood glycemic levels undergoing fixed orthodontic therapy demonstrated changes in the biochemical profiles of GCF and salivary AGEs and proinflammatory cytokine levels in comparison with nondiabetic, healthy subjects.

MATERIALS AND METHODS

Ethical Approval and Study Design

This cross-sectional case-control investigation was conducted according to the guidelines of the Declaration of Helsinki and the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement.[18] The study was submitted, reviewed, and approved by the ethical committee of the King Khalid University College of Dentistry (IRB/KKUCOD/ETR/2023-2024/028). Individuals attending the Department of Orthodontics were selected. A document of informed consent was signed by the individuals willing to participate in the study. In addition, all participants were given a choice to leave the study at any stage without facing any penalty.

Eligibility Criteria

The individuals eligible to be included in this study (1) were prediabetic (HbA1c concentration of $<6.5\%$), had a T2DM diagnosis (HbA1C concentration of $\geq 6.5\%$), and were without a diabetes mellitus diagnosis (HbA1C concentration of $\leq 5.7\%$); (2) were nonsmokers; (3) were aged more than 25 years; (4) were undergoing fixed orthodontic therapy with MBT prescription brackets (0.022-inch brackets and 0.019 $\times$ 0.025-inch stainless steel archwires); and (5) consumed no systemic or topical drugs, including antifungals, antibiotics, and nonsteroidal anti-inflammatory drugs, over the past 3 months. Individuals were excluded if they had undergone any periodontal surgery over the previous 6 months or consumed topical and/or systemic medications.

Diabetic Status

The diagnosis of T2DM was established by assessing serum fasting plasma glucose levels, requiring that the calculated scores were 126 mg/dL (ie, 7.0 mmol/L) without any calorie consumption for 8 hours. The record was further evaluated using glycated hemoglobin (HbA1C) analysis. The participants were considered (1) normal if the HbA1C concentration was $\leq 5.7\%$, (2) prediabetic if the HbA1c concentration was $<6.5\%$, or (3) T2DM if the HbA1C concentration was $\geq 6.5\%$.

Body Mass Index and Sample Size Calculation

Body mass index was measured by dividing the weight of the subjects (kilograms) by height (square meters). The sample size was calculated as previously

described.[13] For each of the individual study groups, (1) nondiabetic, (2) prediabetic, and (3) T2DM, 25 individuals were found to be an adequate sample for achieving 80% power and a 5% alpha level.

Clinical Assessment of Periodontal Parameters

A trained investigator monitored all clinical periodontal parameters. A thorough periodontal assessment was carried out by utilizing a UNC#15 probe (Hu-Friedy, Chicago, Ill). The following clinical periodontal parameters were evaluated: (1) plaque score (PS), (2) probing depth (PD), (3) bleeding on probing, and (4) clinical attachment level. A dichotomous scoring scheme was used to measure the values of BOP and PD as 1 = bleeding/plaque present or 0 = bleeding/plaque absent.

Collection of Gingival Crevicular Fluid and Saliva

GCF and saliva specimens were collected during regular orthodontic visits of participants between 8:00 and 11:00 AM, as described previously.[6] Sampling was performed 8 weeks after the placement of the stainless steel archwires. Saliva collection was performed to determine the rate of flow of resting whole saliva (milliliters per minute). Participants were asked to sit on the dental chair in a relaxed condition, collect saliva in their oral cavity without any movement of muscles, and drool for 5 minutes into a sterile Falcon tube (15 mL).

For GCF collection, supragingival plaque on the mandibular anterior teeth was prudently washed without causing any harm to the soft tissues. For isolation, sterile cotton rolls were utilized. Perio-paper was introduced on the mesial aspect of the gingival sulcus of the mandibular anterior teeth for 30 seconds after the teeth were dried using an air syringe. Samples contaminated with saliva or blood were discarded. The volume of GCF was measured using a Periotron 8000 instrument (OraFlow Inc, Hewlett, NY). The actual volume of the GCF was determined by drawing the results against the flow rate and the standard curve.

Assessment of AGEs and Proinflammatory Chemokines

A quantity of 20 μL of phosphate-buffered saline was incorporated into the Perio strips and the GCF, and centrifugation of the mixture was conducted for 5 minutes at 10,000 rpm. Tumor necrosis factor alpha, IL-6, ghrelin, resistin, AGEs, and receptor activator of nuclear factor κB ligand were evaluated. The measurement of all chemokines was performed using the human magnetic Luminex multiplex assay (R&D Systems Inc, Minneapolis, Minn) according to the manufacturer's instructions. In summary, the amalgamation of the GCF specimens with colored beads was performed, and the specimens were added to a 96-well plate containing analyte coatings. For creating an antigen-antibody sandwich, the addition of biotinylated detection antibodies to the analytes was carried out after the antibodies were adhered to the proteins of the GCF. Next, streptavidin, which had been combined with phycoerythrin, was incorporated for binding with the biotinylated detection antibodies. The reading of the beads was conducted using a dual-laser flow-based detection device (Luminex, Austin, Tex). All biomarkers were denoted in picograms per milliliter and evaluated using standard curves.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) (v.22'IBM Co, Armonk, NY) was used to perform the statistical analysis. Data normalization was performed using the Shapiro-Wilk test, the Kolmogorov-Smirnov test, and Q-Q graphs. The independent *t*-test and the Mann-Whitney *U*-test were carried out for normally and nonnormally distributed data, respectively. The GCF and salivary levels of AGEs, fasting plasma glucose, and HbA1c, along with periodontal clinical parameters, being the descriptive variables, were presented as means ± standard deviations. However, data regarding the GCF and salivary chemokines were presented as means and ranges. The Pearson correlation test was utilized to assess any association between the GCF/salivary chemokine levels and the periodontal clinical parameters. A P value of $<.05$ was considered to be statistically significant.

RESULTS

Table 1 depicts the general characteristics of the study participants. A total of 75 subjects were included. In the nondiabetic group (n = 25), the prediabetic group (n = 25), and the T2DM group (n = 25), the mean ages of the participants were 25.2 years, 28.1 years, and 32.6 years, respectively. Men were more prevalent in all three research groups. There was no statistically significant association between the study groups and the age/sex of the participants ($P > .05$). T2DM group patients had statistically significantly higher levels of HbA1c and fasting plasma glucose ($P < .0001$) and a statistically significantly higher body mass index ($P = .49$) than the prediabetic and nondiabetic patients. Similar oral health practices were conducted and maintained by the participants of all groups since >80% of the subjects reported that they performed toothbrushing 2 times per day.

Table 2 shows the periodontal clinical parameters together with the salivary flow rates of the research groups. A statistically significantly lower mean salivary

Table 1. General Characteristics of the Groups[a]

Characteristic	Value for Group			*P* Value
	Nondiabetic Group	pD Group	T2DM Group	
Number of individuals	25	25	25	–
Age, years (mean ± SD)	25.2 ± 5.1	28.1 ± 4.8	32.6 ± 3.1	.590
Gender (M/F)	15/10	13/12	14/11	.936
HbA1c (mean ± SD)	5.4 ± 1.2	5.9 ± 2.2	7.2 ± 4.3	<.001
BMI (mean ± SD)	20.3 ± 1.5	22.6 ± 4.3	27.0 ± 2.6	.047
FPG (mean ± SD)	95.6 ± 7.2	110 ± 3.2	130.5 ± 6.5	<.0001
Frequency of brushing (%)				
Once a day	8	20	15	.63
Twice a day	92	80	85	

[a] SD indicates standard deviation; M, male; F, female; FPG, fasting plasma glucose; BMI, body mass index; pD, prediabetic; and T2DM, type 2 diabetes mellitus.

flow rate was found among the patients with T2DM than among the prediabetic and nondiabetic patients. Regarding periodontal clinical parameters, only the periodontal BOP scores were found to be significantly higher among T2DM subjects (19.2%) than among nondiabetic (11.2%) and prediabetic (15.9%) subjects. In addition, comparable values were demonstrated by all three study groups regarding PD scores and PSs.

As shown in Table 3, T2DM subjects demonstrated higher scores for GCF chemokines than nondiabetic and prediabetic subjects. A statistically significant difference was found in the levels of AGEs and resistin among the three study groups. The scores for the levels of GCF resistin and AGEs versus periodontal BOP demonstrated a significant positive association by the Pearson correlation test in the T2DM group (Table 4).

DISCUSSION

This study aimed to assess the levels of GCF proinflammatory chemokines and AGEs among nondiabetic, prediabetic, and T2DM subjects together with the influence of diabetes on the chemokine levels of subjects undergoing fixed orthodontic therapy. The present cross-sectional study demonstrated that there were differences in the levels of GCF resistin and AGEs among the participants, with T2DM patients having the highest levels and healthy subjects having the lowest levels. Additionally, among subjects with T2DM, GCF resistin and AGEs showed a high positive association with the periodontal BOP values.

A reduced salivary flow rate was shown in the subjects with T2DM compared to the nondiabetic and prediabetic subjects, which might be explained by the presence of hyposalivation under resting conditions.[19] Low PSs were found in all study groups. The PSs for all research group participants showed the positive impact of toothbrushing while undergoing fixed orthodontic therapy. More than 80% of the study subjects revealed that they performed toothbrushing 2 times per day, which might be a decisive factor associated with the decrease in the PS values. The stringent control of oral health maintenance is imperative during orthodontic therapy since dental plaque readily develops in regions where brushing cannot reach properly and may therefore affect the levels of inflammatory markers.[20]

Residual plaque in contact with fixed orthodontic appliances can cause plaque-related issues, including halitosis, calculus development, and dental caries.[21] The observed proportion of periodontal BOP values was higher in T2DM subjects than in the other two study groups. This may be explained by the presence of increased levels of blood glucose. Investigations have found that higher levels of IL-1β were associated with the formation of a severe periodontal inflammatory response, which is due to persistent hyperglycemia.[22,23]

GCF resistin levels were found to be significantly higher in T2DM participants than in the other two study groups. Resistin is a recently discovered adipocyte

Table 2. Clinical Periodontal Parameters and Unstimulated Whole Salivary Flow Rates[a]

Characteristic	Value for Group			*P* Value
	Nondiabetic Group	pD Group	T2DM Group	
UWSFR, mL/min (mean ± SD)	0.72 ± 0.32	0.60 ± 0.38	0.53 ± 0.12	.048
PS, % (mean ± SD)	10.3 ± 2.8	12.5 ± 3.1	16.1 ± 4.2	.679
BOP, % (mean ± SD)	11.2 ± 3.1	15.9 ± 2.2	19.2 ± 2.8	.042
PD, mm (mean ± SD)	2.2 ± 1.8	2.8 ± 1.5	3.4 ± 2.1	.091

[a] UWSFR indicates unstimulated whole salivary flow rate; PS, plaque score; BOP, bleeding on probing; PD, probing depth; SD, standard deviation; pD, prediabetic; and T2DM, type 2 diabetes mellitus.

Table 3. Proinflammatory Biomarkers and AGEs in the GCF of Study Groups[a]

Variable	Value for Group			*P* Value
	Nondiabetic Group	pD Group	T2DM Group	
GCF flow rate, L/min (mean ± SD)	0.88 ± 0.28	0.92 ± 0.18	0.97 ± 0.54	.427
TNF-α, pg/mL	82.21	95.29	102.45	.754
IL-6, pg/mL	45.67	55.21	64.72	.512
Ghrelin, ng/mL	72.43	80.32	86.34	.945
Resistin, ng/mL	14.21	28.31	36.74	.030
AGEs, pg/mL	215.61	282.29	325.12	.011
RANKL, pg/ml	1095.56	1123.43	1209.71	.256

[a] GCF indicates gingival crevicular fluid; TNF-α, tumor necrosis factor alpha; IL-6, interleukin-6; AGEs, advanced glycation end products; RANKL, receptor activator of nuclear factor κB ligand; pD, prediabetic; T2DM, type 2 diabetes mellitus; and SD, standard deviation.

hormone, and it is involved in potential resistance to insulin. Once assumed to be generated exclusively by adipocytes, resistin is also frequently generated by several immunoinflammatory system cells, suggesting its role in numerous chronic inflammatory diseases and in acting as a proinflammatory cytokine.[24] The increases in the levels of resistin can be explained by the presence of a local proinflammatory condition in periodontal tissues.[25] Hyperglycemia can also be related to increases in the levels of resistin.[26] It has been reported that the composition of subgingival plaque can also be changed in T2DM. This can also be linked with the release of resistin in large amounts from neutrophils.[27] During bone remodeling, osteoclast differentiation is strongly associated with the presence of high levels of GCF resistin.[28]

In T2DM, AGEs play a critical role in the generation of an inflammatory response. AGEs are generated when nucleic acids, lipids, and proteins are oxidized with no utilization of any enzymes.[29] Several investigations have shown that oral and systemic tissues are more likely to produce and aggregate AGEs under chronic hyperglycemic conditions, especially *N*ε-carboxymethyl-lysine.[30,31] The main mechanisms contributing to the induced destruction of AGEs in T2DM can be due to the entrapping and intertwining of protein structures or indirect adherence to cell surface receptors.[32] Even though AGEs can communicate via several receptors, their association with AGE receptors and their role in mediating cellular responses are still understudied.[33] By their association with Toll-like receptors, pattern recognition receptors, G protein–coupled receptors, and scavenger receptors, AGEs may influence cellular processes.[34] The findings of this study showed increased AGE levels among T2DM participants compared to the other two study groups. These results were in agreement with the findings of studies conducted by Alshahrani[6] and Alqerban,[13] who found

Table 4. Pearson Correlation Analysis[a]

Variable	TNF-α	IL-6	Ghrelin	Resistin	AGEs	RANKL
PS						
Nondiabetic group correlation coefficient	−.2652	−.5380	.7021	.7912	−.8248	−.7956
P value	.8821	.1815	.0567	.4113	.5097	.7012
pD group correlation coefficient	.7912	.4367	.5123	.8721	.6231	.6012
P value	.9023	.0834	.0782	.5951	.4398	.3901
T2DM group correlation coefficient	.8812	.4032	.4521	.9371	.4911	.4212
P value	.9867	.0902	.0940	.7253	.3977	.1542
BOP						
Nondiabetic group correlation coefficient	.8196	−.7231	.5632	.8265	−.4748	.8819
P value	.9512	.4267	.0695	.9201	.0612	.5284
pD group correlation coefficient	.7921	.6541	.4921	.6502	.2001	.7981
P value	.8901	.1245	.5732	.7210	.0328	.4182
T2DM group correlation coefficient	−.7233	.9421	.4254	.1172	.0184	.6945
P value	.1287	.1241	.6534	.0210	.0174	.3397
PD						
Nondiabetic group correlation coefficient	.7341	.7134	.4986	.4236	.8971	.4234
P value	.2279	.9839	.9774	.5450	.4561	.5451
pD group correlation coefficient	.8745	.8567	.5845	.3832	.9134	.5025
P value	.3459	.3478	.5624	.7812	.5981	.6974
T2DM group correlation coefficient	.9021	.9567	.7563	.3132	.9476	.9125
P value	.9123	.1453	.3745	.9123	.7563	.8313

[a] TNF-α indicates tumor necrosis factor alpha; IL-6, interleukin-6; AGEs, advanced glycation end products; RANKL, receptor activator of nuclear factor κB ligand; PS, plaque score; BOP, bleeding on probing; PD, pocket depth; pD, prediabetic; and T2DM, type 2 diabetes mellitus.

that the increased levels of proinflammatory cytokines among T2DM subjects were contributing to soft and hard tissue problems. The increased AGE levels in chronic hyperglycemia might aggravate the proinflammatory response, destroying the soft tissue and alveolar bone and therefore further impacting OTM.[35]

During fixed orthodontic therapy, therefore, it is very important for T2DM patients to pay greater attention to the maintenance of good oral hygiene. T2DM subjects undergoing orthodontic therapy might experience increased levels of periodontal infections, higher levels of chemokines, and periodontal degeneration.

CONCLUSIONS

- T2DM patients demonstrated significantly higher levels of GCF resistin and AGEs during fixed orthodontic therapy. Chronically hyperglycemic patients undergoing orthodontic therapy were shown to have a proinflammatory response.

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