Original Article

Levels of proinflammatory chemokines and advanced glycation end products in patients with type-2 diabetes mellitus undergoing fixed orthodontic treatment

Ali Alqerban[a]

ABSTRACT

Objectives: To explore the effect of diabetic status on the proinflammatory chemokine profile and levels of advanced glycation end products (AGEs) in gingival crevicular fluid (GCF) derived from patients with type 2 diabetes mellitus (T2DM) undergoing fixed orthodontic treatment.

Materials and Methods: Two groups, nondiabetic and T2DM, were included. Their demographics, biochemical parameters including hemoglobin A1c, fasting blood glucose, body mass index, and oral hygiene status were recorded. GCF sampling was performed after 2 months of placement of stainless steel archwires and chemokines (primary outcome) were quantified using Human Magnetic Luminex multiplex assay. Secondary outcomes were assessment of clinical periodontal status, unstimulated whole saliva flow rate, and GCF flow rate.

Results: Twenty participants each in the nondiabetic (mean age: 26.4 years) and T2DM (mean age: 27.2 years) groups gave consent to participate. Biochemical analysis showed significantly increased values for hemoglobin A1c ($P < .001$), fasting blood glucose ($P < .0001$), body mass index($P = .047$), and unstimulated whole saliva flow rate ($P = .041$) for the T2DM group. Bleeding on probing was significantly higher in participants with T2DM than nondiabetic participants ($P = .039$). Resistin ($P = .034$) and AGEs ($P = .017$) showed significantly higher values in participants with T2DM than nondiabetic participants. Pearson correlation analysis indicated a significant positive correlation between resistin ($r = 0.1372$; $P = .022$) and AGEs ($r = 0.0194$; $P = .0186$) against bleeding on probing in participants with T2DM.

Conclusions: The biochemical profile of GCF samples from participants with T2DM after alignment demonstrated higher levels of resistin and AGEs. The data suggest the presence of a proinflammatory response in patients with T2DM undergoing fixed orthodontic treatment. (*Angle Orthod.* 2021;91:105–110.)

KEY WORDS: Type 2 diabetes mellitus; Advanced glycation end products; Resistin; Gingival crevicular fluid; Orthodontics

INTRODUCTION

Type 2 diabetes mellitus (T2DM) has become a leading community health problem and has almost quadrupled in the past three and a half decades. Now the ninth major cause of mortality, T2DM has become progressively prevalent in the Western population.[1] It is manifested by chronic hyperglycemia where the body becomes resistant to the normal effects of insulin or has gradual decrease in insulin production.[2] Research suggests that the pathogenesis of T2DM links the disease to a state of subclinical chronic inflammation.[3] In the chronic hyperglycemic state, several proteins undergo glycosylation that subsequently leads to the accumulation of advanced glycation end products (AGEs) in the soft tissues.[4] AGEs cross-link collagen by making them less soluble with minimum reparative tendency for periodontal tissues.[4] Therefore, increased levels

[a] Associate Professor, Department of Preventive Dental Science, College of Dentistry, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia; and Department of Preventive Dental Science, College of Dentistry, Dar Al Uloom University, Riyadh, Saudi Arabia.

Corresponding author: Dr Ali Alqerban, Department of Preventive Dental Science, College of Dentistry, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia (email: a.alqerban@psau.edu.sa)

Accepted: July 2020. Submitted: May 2020.
Published Online: October 26, 2020

© 2021 by The EH Angle Education and Research Foundation, Inc.

of AGEs with other altered cellular responses renders the periodontal structures susceptible to periodontal breakdown.[5]

Orthodontic tooth movement is a coordinated remodeling of the soft tissue within the periodontal ligament and surrounding bone that follows a localized aseptic inflammatory response driven by external force.[6] This culminates in osteoclastic activity in compression areas, while the zone of tension involves the deposition of osteoblasts. A series of secreted factors are known to be expressed during orthodontic tooth movement, and many of these are detectable in the gingival crevicular fluid (GCF).[7] These include such inflammatory biomarkers as tissue necrosis factors[8] and interleukins (ILs);[9] the connective tissue-remodeling biomarkers, including matrix metalloproteinase-8 and matrix metalloproteinase-9[10]; and the bone-resorptive biomarkers, such as receptor activator of nuclear factor kappa-B ligand (RANKL).[11] Given the relation between the presence of chronic systemic inflammation and a raised hemoglobin A1c (HbA1c), the potential implications for periodontal disease in patients with T2DM are well-established.[12] Individuals with T2DM exhibit impaired levels of proinflammatory cytokines in the presence of periodontal disease[13] and, therefore, have a higher risk of developing periodontitis[14] with worse periodontal therapy outcomes.[15]

Interestingly, the effect of diabetes on orthodontic tooth movement has widely been studied in rat models.[16,17] These studies produced conflicting results showing increased rate of orthodontic tooth movement,[17] while other studies suggested reduced tooth movement.[16] However, no clinical trials or cross-sectional cohort studies have been performed in humans that assessed the rate of tooth movement in diabetic status. The molecular mechanism implications of diabetic status for orthodontic therapy explains the alteration in enzymes and proinflammatory factors involved in osteoclastic and osteoblastic activity.[7,18] Just as orthodontic tooth movement has been studied in individuals with obesity,[19] which focused on the concept that subtle proinflammatory changes are contained in the periodontal structure of individuals who are obese might significantly affect rates of tooth movement, the current study speculated the same phenomenon among individuals with diabetes. It was hypothesized that T2DM may alter the biochemical status of GCF of patients undergoing fixed orthodontic treatment. Therefore, the aim of the present study was to explore the effect of diabetic status on the proinflammatory chemokine profile and levels of AGEs in GCF derived from patients with T2DM undergoing fixed orthodontic treatment.

MATERIALS AND METHODS

Ethics and Setting

This cross-sectional case control study followed the STROBE guidelines[20] and was carried out following the principles stated in the Declaration of Helsinki. Ethical approval was obtained from the Scientific Research Unit of Dar Al Uloom University, Riyadh, Saudi Arabia in November 2019. The Institutional Review Board approved the study under approval number REC009/2019. Patients were recruited from the Department of Orthodontics, Dar Al Uloom University, Riyadh, Saudi Arabia. Written informed consent that described the purpose of the study protocol was signed by all participating patients, and they were permitted to withdraw from the study at any stage without any consequences.

Patients

Patients for this study were recruited if they met the following inclusion criteria: (1) nonsmoker, (2) age range 25–55 years, (3) medically diagnosed T2DM (according to the American Diabetes Association 2020)[21] or no diagnosis of diabetes, (4) undergoing fixed orthodontic therapy using MBT prescription 0.022-inch brackets (3M-Unitek, Monrovia, CA) and 0.019×0.025-inch stainless steel archwire for at least 4 weeks, and (5) using no nonsteroidal anti-inflammatory or systemic antimicrobial drugs in the previous 6 months.

The presence of T2DM was confirmed through serum fasting plasma glucose (FPG) values that recorded $\geq$126 mg/dL or 7.0 mmol/L with no caloric intake for at least 8 hours. The record was further confirmed by performing HbA1C test that gave results $\geq$6.5% or 48 mmol/mol. These tests were performed using a certified National Glycohemoglobin Standardization Program and standardized Diabetes Control and Complications Trial assay.[22] Body mass index (BMI) was measured by recording the weight of the patients in kilograms and dividing by height in meters squared.

Power Analysis

Power analysis was based on a previous study investigating GCF chemokines in obesity.[23] To recognize a significant difference in crevicular fluid chemokines between those with T2DM and nondiabetic individuals, the sample size of 20 participants in each group would be sufficient to obtain 80% power and keep the alpha level at 5%.

Clinical Periodontal Health Parameters

Clinical periodontal health parameters, including plaque scores (PSs), bleeding on probing (BOP), and probing depth (PD), were measured around all teeth using a manual periodontal probe (UNC-15, Hu-Friedy, Chicago, IL).[24] PSs and BOP were recorded using a dichotomous scoring system: 1 = plaque/bleeding present, 0 = plaque/bleeding absent. PD was estimated by recording the millimeter markings from the gingival margin to the base of the gingival sulcus.

Sample Collection

Saliva and GCF samples were collected in the morning between 8 AM and 11 AM during routine orthodontic appointments. Sampling was performed 2 months after placement of stainless steel archwires. Saliva was pooled to assess only the unstimulated whole saliva flow rate (UWSFR) in milliliters per minute. Patients were asked to sit comfortably on the dental chair, pool saliva in their mouth without any muscle movement, and drool the saliva into a sterile 15-mL falcon tube for 5 minutes. To collect GCF, lower anterior teeth were isolated from saliva with cotton rolls, and supragingival plaque was carefully removed without injuring the soft tissue. After drying the teeth with an air-syringe, six Perio papers (Oraflow Inc., Amityville, NY, USA) were inserted in the mesial gingival sulcus of all lower anterior teeth for 30 seconds and pooled. Samples contaminated with saliva or blood were discarded. The volume of GCF was analyzed using a Periotron 8000 electronic measuring device (OraFlow Inc) and the recordings plotted against the standard curve and flow rate to detect the actual volume.

Laboratory Quantification of Proinflammatory Chemokines and AGEs

The collected GCF along with Perio strips were transferred in 20 μL of phosphate buffered saline and the mixture centrifugated at 10,000 rpm for 5 minutes. GCF was analyzed for proinflammatory cytokines (tumor necrosis factor-α and IL-6), inflammatory adipokines (ghrelin and resistin), diabetic chemokine (AGEs), and bone-remodeling chemokine (RANKL) all expressed in pg/mL. Kappa scores were recorded to assess reliability of the single assessor that showed excellent agreement (κ = 0.87). All chemokines were quantified using Human Magnetic Luminex multiplex assay (R&D Systems, Inc. Minneapolis, MN). The protocol was followed according to the guidelines recommended in the manufacturing kit. In short, GCF samples were mixed with color-coded beads and added to 96-well microplates already coated with analyte-specific capture antibodies. After binding of antibodies with GCF proteins, biotinylated detection antibodies specific to analytes were added to form an antibody-antigen sandwich. Subsequently, phycoerythrin-conjugated streptavidin was added to bind the biotinylated detection antibodies. Beads were read on a dual-laser flow-based detection instrument (Luminex, Austin, TX, USA).

Table 1. General Characteristics of the Study Groups[a]

Characteristics	Nondiabetic Group	T2DM Group	*P*-Value[b]
Total number of patients	20	20	
Age (mean ± SD)	26.4 ± 7.1	27.2 ± 6.9	.582
Gender (male/female)	7/13	9/11	.914
HbA1c (mean ± SD)	5.1 ± 1.7	7.5 ± 0.9	**<.001**
FPG (mean ± SD)	96.6 ± 8.2	129 ± 4.9	**<.0001**
Body mass index (mean ± SD)	21.8 ± 1.3	26.7 ± 2.0	**.047**
Brushing frequency (%)			
Once a day	9	16	.063
Twice a day	91	84	

[a] FPG indicates fasting plasma glucose; HbA1c, hemoglobin A1c; SD, standard deviation; T2DM, type 2 diabetes mellitus.

[b] *P*-values determined using independent *t*-test. Bold numbers indicate statistically significant difference between groups at $P < .05$.

Data Analysis

All data were recorded on a Microsoft Excel sheet and later transferred in specialized SPSS statistics software (v.21, IBM Co, Armonk, NY) for statistical analyses. Normality testing was performed using Shapiro-Wilk and Kolmogorov-Smirnov tests and later confirmed through plotting Q-Q graphs before estimating the *P*-value for all variables. Statistical significance was set at .05. For normal distribution, an independent *t*-test was performed or the Mann-Whitney *U*-test was computed for nonnormally distributed data. Descriptive variables, including age, HbA1c and FPG levels, BMI, and clinical periodontal parameters, were reported as means and standard deviations, while data for GCF chemokines were reported as means and range. Pearson correlation analysis was performed for any correlation between clinical periodontal parameters and GCF chemokine levels.

RESULTS

Twenty participants each in the nondiabetic (mean age = 26.4 years) and T2DM (mean age = 27.2 years) groups gave consent to participate. There were more women than men in both groups. Age and gender were not significantly different between the groups ($P > .05$). Biochemical analysis showed statistically significant increased values for HbA1c ($P < .001$), FPG ($P < .0001$), and BMI ($P = .047$) for the T2DM group compared with the nondiabetic group. Oral hygiene

Table 2. UWSFR and Full-Mouth Clinical Periodontal Parameters of the Study Groups [a]

Characteristics	Nondiabetic Group	T2DM Group	*P*-Value[b]
UWSFR, mL/min (mean ± SD)	0.76 ± 0.42	0.52 ± 0.23	**.041**
Plaque scores, % (mean ± SD)	11.4 ± 3.7	15.1 ± 4.8	.633
Bleeding on probing, % (mean ± SD)	10.5 ± 3.2	18.9 ± 2.4	**.039**
Probing depth, mm (mean ± SD)	2.8 ± 1.1	3.3 ± 2.0	.089

[a] SD, standard deviation; T2DM, type 2 diabetes mellitus; UWSFR, unstimulated whole saliva flow rate.
[b] *P*-value determined using independent *t*-test. Bold numbers indicate statistically significant difference between groups at $P < .05$.

care was comparable in both groups; more than 80% reported brushing twice daily (Table 1).

Unstimulated whole saliva flow rate and full-mouth clinical periodontal parameters for the study groups are reported in Table 2. The mean UWSFR in patients with T2DM was significantly lower than for nondiabetic participants ($P = .041$). Among clinical periodontal parameters, only percentage of BOP was significantly higher in participants with T2DM (18.9%) than nondiabetic participants (10.5%) ($P = .039$). The mean PS and PD was comparable between nondiabetic patients and patients with T2DM.

The results for GCF chemokines showed a general trend of higher values in patients with T2DM than nondiabetic patients. However, only two showed significant differences: resistin ($P = .034$) and AGEs ($P = .017$) showed significantly higher values in patients with T2DM than nondiabetic patients (Table 3). Pearson correlation analysis for individual groups indicated a significant positive correlation between GCF resistin ($r = 0.1372$; $P = .022$) and AGEs ($r = 0.0194$; $P = .0186$) levels against BOP in the T2DM group (Table 4).

DISCUSSION

The present study was a novel study in humans that aimed to explore the levels of GCF proinflammatory chemokines and levels of AGEs in patients with T2DM and nondiabetic patients and to explore the effect of diabetic status on the chemokine profiles derived from patients undergoing fixed orthodontic treatment. The results of this study indicated that levels of resistin and AGEs in the GCF were significantly higher in patients with T2DM than nondiabetic patients. In addition, these two chemokines showed strong positive correlation with bleeding scores among patients with T2DM.

This cross-sectional study was carefully designed to include matched cohorts for both T2DM and nondiabetic groups. However, the biochemical variables could not be matched due to the inclusion of type of cohort with an exposure (diabetic status). It is noteworthy that the BMI of the T2DM group was significantly higher than the BMI for the nondiabetic group. The mean BMI for the T2DM group was 26.7, which is normally considered overweight and not obese. A recent cross-sectional study indicated an increase of metabolic leptin and tissue remodeling chemokines in patients who are obese, which suggested an increased proinflammatory status in the periodontium of patients with obesity who reported in the final stages of orthodontic treatment.[23] Future cross-sectional studies should be undertaken to evaluate the combined effect of obesity and T2DM on the GCF chemokines in patients undergoing fixed orthodontic treatment.

The T2DM group demonstrated a low salivary flow rate compared with the nondiabetic group. The significantly reduced UWSFR in the T2DM cohort may be explained by the presence of hyposalivation at rest in patients with T2DM compared with systemically healthy patients.[25] Furthermore, it is well established that stringent oral hygiene care is associated with low amounts of microbial plaque.[26] This is routinely practiced in orthodontics treatment during which patients with fixed orthodontic devices are given strict oral hygiene measures to reduce microbial plaque around gingival tissues. The low values of plaque scores in the cohort groups reflected the frequency of twice daily toothbrushing practiced by more than 80% of the participants in both groups. However, the percentage of bleeding was significantly higher among

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

Variables	Nondiabetic Group	T2DM Group	*P*-Value[b]
GCF flow rate, μL/min	0.93 ± 0.38	0.97 ± 0.45	.417
TNF-α, pg/mL (range)	85.61 (66.91–92.73)	101.42 (89.65–114.27)	.737
IL-6, pg/mL (range)	47.18 (38.05–55.14)	61.98 (49.82–72.47)	.507
Ghrelin, ng/mL (range)	76.52 (61.07–88.21)	84.90 (79.58–99.72)	.923
Resistin, ng/mL (range)	15.52 (12.76–17.32)	34.02 (29.41–38.54)	**.034**
AGEs, pg/mL (range)	228.42 (192.45–269.54)	381.28 (340.81–411.57)	**.017**
RANKL, pg/mL (range)	1188.13 (1095.73–1165.65)	1268.72 (1195.84–1342.44)	.248

[a] AGE indicates advanced glycation end product; GCF, gingival crevicular fluid; IL, interleukin; RANKL, receptor activator of nuclear factor kappa-B ligand; T2DM, type 2 diabetes mellitus; TNF, tumor necrosis factor.
[b] *P*-value determined using Mann-Whitney *U*-test. Bold numbers indicate statistically significant difference between groups at $P < .05$.

Table 4. Pearson Correlation Analysis Between Clinical Periodontal Parameters and GCF Biomarkers in Nondiabetic and T2DM Groups[a,b]

Variables	TNF-α	IL-6	Ghrelin	Resistin	AGEs	RANKL
Plaque scores						
Nondiabetic group correlation coefficient	–0.2754	–0.5490	0.7513	0.7912	–0.8356	–0.8210
P-value	.8923	.1845	.0604	.4113	.5186	.7012
T2DM group correlation coefficient	0.8911	0.3985	0.4733	0.9371	0.5011	0.4242
P-value	.9936	.0923	.0951	.7253	.4277	.1598
Bleeding on probing						
Nondiabetic group correlation coefficient	0.8219	–0.7511	0.5732	0.8162	–0.4848	0.8819
P-value	.9615	.4395	.0701	.9110	.0644	.5284
T2DM group correlation coefficient	–0.7322	0.3941	0.4353	0.1372	0.0194	0.6936
P-value	.1348	.0856	.6672	**.0226**	**.0186**	.3487
Probing depth						
Nondiabetic group correlation coefficient	0.7553	0.7346	0.4986	0.4857	0.9536	0.4534
P-value	.2364	.9786	.9774	.5698	.4564	.5675
T2DM group correlation coefficient	0.9234	0.9483	0.7563	0.3874	0.9484	0.9487
P-value	.9546	.1345	.3745	.9867	.7573	.8374

[a] AGE indicates advanced glycation end product; GCF, gingival crevicular fluid; IL, interleukin; RANKL, receptor activator of nuclear factor kappa-B ligand; T2DM, type 2 diabetes mellitus; TNF, tumor necrosis factor.
[b] Bold letters indicate statistically significant difference between groups at $P < .05$.

patients with T2DM than among nondiabetic patients. This may be attributed to the presence of heightened glycemic levels. Research indicated that chronic hyperglycemia contributes to the development of a profound inflammatory response around the periodontal tissues, which is associated with elevated levels of the GCF proinflammatory biomarker IL-1β.[27]

Patients with T2DM showed significantly higher levels of resistin in GCF than nondiabetic patients. This could be due to the heightened expression of resistin by the local proinflammatory state in the periodontium, as well as a chromic systemic inflammatory state owing to hyperglycemia.[28] In addition, it is suggested that T2DM may qualitatively alter the periodontal microbial plaque composition in the subgingival plaque.[29] In this regard, resistin may also be released from inflammatory neutrophils by putative periodontal bacteria.[30,31] Increased resistin levels may also correspond with differentiation of osteoclasts, which suggests an important role for resistin in bone remodeling.[32] Similarly, the raised levels of AGEs in high glycemic conditions may exaggerate the inflammatory response and cause destruction of alveolar bone.[5] In addition, higher levels of AGEs may contribute to the increased level of soft tissue destruction in T2DM.[33] However, the role of AGEs and resistin in orthodontic soft tissue and bone remodeling is still unclear; therefore, future prospective studies should be carried out to assess the levels of AGEs and resistin during the alignment phase of orthodontic treatment.

Therefore, patients with T2DM should be extremely cautious in maintaining oral hygiene during fixed orthodontic treatment. T2DM may modulate the periodontal pathogens and may lead to additional periodontal deterioration and, hence, elevated chemokine levels in patients undergoing orthodontic treatment.

CONCLUSIONS

- The biochemical profile of GCF samples from patients with T2DM after alignment demonstrated significantly higher levels of resistin and AGEs. These data suggest the presence of a proinflammatory response in patients with T2DM undergoing fixed orthodontic treatment.

ACKNOWLEDGMENT

The authors extend their appreciation to the Deanship of Postgraduate Studies and Scientific Research at Dar Al Uloom University for funding this work.

REFERENCES

1. DeFronzo RA, Ferrannini E, Groop L, et al. Type 2 diabetes mellitus. *Nat Rev Dis Primers*. 2015;1:1–22.
2. Association AD. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2014;37:S81–S90.
3. Gokulakrishnan K, Mohanavalli KT, Monickaraj F, Mohan V, Balasubramanyam M. Subclinical inflammation/oxidation as revealed by altered gene expression profiles in subjects with impaired glucose tolerance and type 2 diabetes patients. *Mol Cell Biochem*. 2009;324:173–181.
4. Katz J, Bhattacharyya I, Farkhondeh-Kish F, Perez F, Caudle R, Heft M. Expression of the receptor of advanced glycation end products in gingival tissues of type 2 diabetes patients with chronic periodontal disease: a study utilizing immunohistochemistry and RT-PCR. *J Clin Periodontol*. 2005;32:40–44.
5. Akram Z, Alqahtani F, Alqahtani M, Al-Kheraif AA, Javed F. Levels of advanced glycation end products in gingival crevicular fluid of chronic periodontitis patients with and without type-2 diabetes mellitus. *J Periodontol*. 2020;91:396–402.

6. Li Y, Jacox LA, Little SH, Ko C-C. Orthodontic tooth movement: the biology and clinical implications. *Kaohsiung J Med Sci*. 2018;34:207–214.
7. Nunes L, Quintanilha L, Perinetti G, Junior JC. Effect of orthodontic force on expression levels of ten cytokines in gingival crevicular fluid. *Arch Oral Biol*. 2017;76:70–75.
8. Bletsa A, Berggreen E, Brudvik P. Interleukin-1α and tumor necrosis factor-α expression during the early phases of orthodontic tooth movement in rats. *Eur J Oral Sci*. 2006; 114:423–429.
9. Leethanakul C, Suamphan S, Jitpukdeebodintra S, Thongudomporn U, Charoemratrote C. Vibratory stimulation increases interleukin-1 beta secretion during orthodontic tooth movement. *Angle Orthod*. 2016;86:74–80.
10. Ingman T, Apajalahti S, Mäntylä P, Savolainen P, Sorsa T. Matrix metalloproteinase-1 and-8 in gingival crevicular fluid during orthodontic tooth movement: a pilot study during 1 month of follow-up after fixed appliance activation. *Eur J Orthod*. 2005;27:202–207.
11. Yamaguchi M. RANK/RANKL/OPG during orthodontic tooth movement. *Orthod Craniofac Res*. 2009;12:113–119.
12. Alasqah M, Mokeem S, Alrahlah A, et al. Periodontal parameters in prediabetes, type 2 diabetes mellitus, and non-diabetic patients. *Braz Oral Res*. 2018;32:e81.
13. Genco RJ, Grossi SG, Ho A, Nishimura F, Murayama Y. A proposed model linking inflammation to obesity, diabetes, and periodontal infections. *J Periodontol*. 2005;76:2075–2084.
14. Jimenez M, Hu FB, Marino M, Li Y, Joshipura KJ. Type 2 diabetes mellitus and 20 year incidence of periodontitis and tooth loss. *Diabetes Res Clin Pract*. 2012;98:494–500.
15. Artese HPC, Foz AM, de Sousa Rabelo M, et al. Periodontal therapy and systemic inflammation in type 2 diabetes mellitus: a meta-analysis. *PLoS One*. 2015;10:e0128344.
16. Arita K, Hotokezaka H, Hashimoto M, et al. Effects of diabetes on tooth movement and root resorption after orthodontic force application in rats. *Orthod Craniofac Res*. 2016;19:83–92.
17. Braga SM, de Albuquerque Taddei SR, Andrade I Jr, et al. Effect of diabetes on orthodontic tooth movement in a mouse model. *Eur J Oral Sci*. 2011;119:7–14.
18. Kapoor P, Monga N, Kharbanda OP, Kapila S, Miglani R, Moganty R. Effect of orthodontic forces on levels of enzymes in gingival crevicular fluid (GCF): a systematic review. *Dent Press J Orthod*. 2019;24:40.e41–40.e22.
19. Michelogiannakis D, Rossouw PE, Khan J, Akram Z, Menenakos E, Javed F. Influence of increased body mass index on orthodontic tooth movement and related parameters in children and adolescents: a systematic review of longitudinal controlled clinical studies. *J Orthod*. 2019;46: 323–334.
20. Von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann Intern Med*. 2007;147:573–577.
21. American Diabetes Association. 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2020. *Diabetes Care*. 2020;43:S14–S31.
22. Little RR. Glycated hemoglobin standardization—National Glycohemoglobin Standardization Program (NGSP) perspective. *Clin Chem Lab Med*. 2003;41:1191–1198.
23. Saloom HF, Carpenter GH, Cobourne MT. A cross-sectional cohort study of gingival crevicular fluid biomarkers in normal-weight and obese subjects during orthodontic treatment with fixed appliances. *Angle Orthod*. 2019;89:930–935.
24. Akram Z, Abduljabbar T, Hosain M, et al. Comparison of periodontal inflammatory parameters among habitual gutka-chewers and naswar-dippers: a split-mouth retrospective clinical study. *Acta Odontol Scand*. 2018;76:141–147.
25. Carramolino-Cuéllar E, Lauritano D, Silvestre FJ, Carinci F, Lucchese A, Silvestre-Rangil J. Salivary flow and xerostomia in patients with type 2 diabetes. *J Oral Pathol Med*. 2018;47: 526–530.
26. Feres M, Gursky LC, Faveri M, Tsuzuki CO, Figueiredo LC. Clinical and microbiological benefits of strict supragingival plaque control as part of the active phase of periodontal therapy. *J Clin Periodontol*. 2009;36:857–867.
27. Engebretson SP, Hey-Hadavi J, Ehrhardt FJ, et al. Gingival crevicular fluid levels of interleukin-1β and glycemic control in patients with chronic periodontitis and type 2 diabetes. *J Periodontol*. 2004;75:1203–1208.
28. Gokhale NH, Acharya AB, Patil VS, Trivedi DJ, Setty S, Thakur SL. Resistin levels in gingival crevicular fluid of patients with chronic periodontitis and type 2 diabetes mellitus. *J Periodontol*. 2014;85:610–617.
29. Zhou M, Rong R, Munro D, et al. Investigation of the effect of type 2 diabetes mellitus on subgingival plaque microbiota by high-throughput 16S rDNA pyrosequencing. *PLoS One*. 2013;8:e61516.
30. Hiroshima Y, Bando M, Inagaki Y, et al. Resistin in gingival crevicular fluid and induction of resistin release by porphyromonas gingivalis lipopolysaccharide in human neutrophils. *J Periodontal Res*. 2012;47:554–562.
31. Furugen R, Hayashida H, Saito T. Porphyromonas gingivalis and Escherichia coli lipopolysaccharide causes resistin release from neutrophils. *Oral Dis*. 2013;19:479–483.
32. Thommesen L, Stunes AK, Monjo M, et al. Expression and regulation of resistin in osteoblasts and osteoclasts indicate a role in bone metabolism. *J Cell Biochem*. 2006;99:824–834.
33. Ilea A, Băbţan AM, Boşca BA, et al. Advanced glycation end products (AGEs) in oral pathology. *Arch Oral Biol*. 2018;93: 22–30.