Original Article

A cross-sectional cohort study of gingival crevicular fluid biomarkers in normal-weight and obese subjects during orthodontic treatment with fixed appliances

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ABSTRACT

Objectives: To investigate the effects of obesity on biomarker levels within lower incisor gingival crevicular fluid (GCF) in subjects undergoing routine fixed appliance orthodontic treatment.

Materials and Methods: This was a cross-sectional clinical cohort study. GCF was collected from normal-weight and obese subjects at completion of alignment at least 1 month after placement of 0.019 × 0.025-inch stainless-steel archwires. The primary outcome was the difference in GCF biomarker levels between groups. Secondary outcomes included differences in clinical parameters of plaque and gingival indices, unstimulated whole-mouth saliva, and GCF flow rates.

Results: Thirty-eight subjects (18 male, 20 female) with a mean age of 25.6 (SD, 6.3) years and mean body mass index (BMI) of 22.6 (1.6) in normal-weight and 32.4 (2.2) kg/m^2 in obese groups were investigated. Apart from BMI ($P < .0001$), there were no statistically significant differences in essential demographics between groups. Significantly increased levels of the adipokine leptin ($P < .009$) and the tissue-remodeling biomarker matrix metalloproteinase-9 (MMP9; $P < .020$) were identified in the obese cohort. For the remainder of the biomarkers, including the RANKL bone-remodeling marker and several inflammatory markers, there were no significant differences between groups. No correlation was observed between plaque index or gingival index for any GCF biomarker for either group ($P = .07–1.00$).

Conclusions: This study investigated the GCF biochemical profile of obese and normal-weight subjects undergoing fixed-appliance orthodontic treatment. Significantly increased levels of the adipokine leptin and the tissue-remodeling biomarker MMP9 were identified in the obese group. These data provide evidence of differences in GCF biochemistry between obese and normal-weight subjects undergoing fixed appliance orthodontic treatment. (*Angle Orthod.* 2019;89:930–935.)

KEY WORDS: Obesity; Orthodontics; Biomarkers; GCF

INTRODUCTION

Obesity has become increasingly common in Western societies over the past few decades,[1] which has major implications for health care because of the known associations between raised body mass index (BMI) and multiple chronic diseases, including diabetes, cardiovascular disease, and cancer.[2] Obesity represents a state of chronic inflammation mediated through the presence of excess adipose tissue.[3] Adipocytes are the predominant cellular component of adipose tissue and are now recognized as producers of multiple metabolically active proteins or adipokines that can influence systemic metabolic function and inflammation.[4] These include the proinflammatory adipokines leptin[5] and resistin[6] and the anti-inflammatory adiponectin.[7]

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Orthodontic tooth movement occurs through connective tissue remodeling within the periodontium and alveolar bone following a localized inflammatory reaction induced by external force. A host of biochemical mediators are known to be induced during this process, and many of these are detectable in the gingival crevicular fluid (GCF),[8] including the inflammatory mediators myeloperoxidase (MPO)[9,10] and C-reactive protein (CRP)[11]; the tissue-remodeling biomarkers matrix metalloproteinase-8 (MMP8), matrix metalloproteinase-9 (MMP9), and tissue inhibitor of metalloproteinase 1 (TIMP1); and the bone-remodeling biomarker receptor activator of nuclear factor kappa-B ligand (RANKL).[12] Given the associations between a raised BMI and the presence of chronic systemic inflammation, there are potential implications for periodontal health in the obese individual. Obese subjects demonstrate variation in inflammatory markers in the presence of periodontal disease[13] have an increased risk of chronic periodontitis[14,15] and poorer response to nonsurgical periodontal therapy.[16] More recently, orthodontic tooth movement has been investigated in obese subjects, focusing on the theory that proinflammatory change in the periodontium of obese subjects might influence rates of tooth movement. Interestingly, in a cohort of subjects undergoing treatment with fixed appliances, initial tooth displacement was significantly increased in the obese group and, after adjustment for confounders, obese subjects had a higher rate of tooth movement compared with subjects of normal weight.[17] A host of further implications of obesity for orthodontic treatment has also been discussed, including psychosocial well-being in these individuals, variation in pubertal and craniofacial growth patterns, and stability of treatment.[18,19] Increased BMI also appears to be a risk factor for less cooperation with removable and fixed appliances, longer treatment duration, and more oral health–related problems during fixed appliance treatment.[20,21]

The aim of this cross-sectional study was to investigate the influence of a raised BMI on the biochemical profile of GCF derived from subjects undergoing orthodontic treatment with fixed appliances.

MATERIALS AND METHODS

Study Design

This cross-sectional cohort study compared the effects of obesity on selected biomarkers within GCF in subjects undergoing routine orthodontic treatment with fixed appliances. Ethical approval was obtained from the United Kingdom National Research Ethics Service (14/LO/0769), and written informed consent was received from all subjects. Data are reported according to STROBE guidelines.[22]

Setting

Participants were recruited from the orthodontic treatment clinic at King's College London Dental Institute (Guy's Hospital), United Kingdom.

Participants

Participants were purposively recruited with matched age and gender into this cohort study according to the following criteria: (1) 18–45 years old, (2) undertaking fixed appliance orthodontic treatment (3M Victory-APC 0.022-inch brackets, MBT prescription, 3M-Unitek, Monrovia, Calif) and in 0.019 × 0.025-inch stainless-steel archwires for at least 1 month, (3) no medical contraindications or regular medication (including antibiotic therapy) in the previous 6 months, (4) nonsmoking, and (5) normal weight (BMI = 18.5–24.9) and obese (BMI $\geq$30) classification. Those classified as underweight (BMI $<$18.5) and overweight (BMI = 25–29.9) were excluded.

Subject body weight was measured to the nearest 0.1 kg using a calibrated scale and height measured to the nearest centimeter using a wall-mounted ruler. The BMI was calculated as mass (kg) divided by height in meters squared (kg/m^2). All measurements were taken by a single-trained operator (Dr Saloom) using the same equipment.

Variables

Sample collection took place during a routine orthodontic appointment (between 9:30 AM and 3:30 PM) at least 1 month after placement of 0.019 × 0.025-inch stainless-steel archwires to ensure a passive fit of the archwire and therefore completion of alignment. Unstimulated whole-mouth salivary flow rate (uWMS) was calculated as milliliters per minute from saliva obtained from relaxed patients by drooling into a plastic tube for 5 minutes. Periodontal health was measured clinically using validated plaque and gingival indices.[23,24] GCF was collected once from the distal side of the lower six anterior teeth (canine to canine) and pooled. Teeth were isolated and gently dried using an air syringe. Filter-paper strips (Periopaper, OraFlow Inc, New York, NY) were then placed 1 mm into the gingival crevice for 30 seconds. If there was any contamination of the strip with saliva or blood, it was discarded. The volume of collected fluid in the strip was measured directly using a Periotron 8000 electronic micro-moisture meter (OraFlow Inc) with readings converted to an actual volume by reference to the standard curve and flow rate calculated (per minute).

Table 1. Demographics of Subjects[a]

Demographics	Overall	Normal Weight	Obese	*P* Value
Patients, n	38	19	19	
Male/female, n	18/20	9/10	9/10	
Age, y	25.6 (6.3)	24.6 (6.5)	26.6 (6.2)	.338[b]
BMI, kg/m^2)	27.5 (5.3)	22.6 (1.6)	32.4 (2.2)	**<.0001**[b]
uWMS, mL/min	0.67 (0.28)	0.68 (0.31)	0.65 (0.26)	.73[b]
Plaque index	1.3 (0.5)	1.26 (0.42)	1.33 (0.50)	.662[b]
Gingival index	2.0 (0.3)	1.94 (0.29)	2.04 (0.28)	.302[b]

[a] For demographics, values are mean (SD). Significant results are indicated in bold. BMI indicates body mass index; uWMS, unstimulated whole-mouth salivary flow rate.
[b] Independent *t*-test.

GCF was retrieved from filter strips with the addition of 20 μL phosphate-buffered saline and centrifugation for 5 minutes at 9200*g*. GCF was analyzed by Luminex magnetic bead-based multiplex assay using a commercial kit (R&D systems, Abingdon, UK) for detection (in ng/mL, except where indicated) of the adipokines adiponectin, leptin (pg/mL), and resistin; the inflammatory mediators MPO and CRP; the tissue-remodeling biomarkers MMP8, MMP9, TIMP1, and the MMP8/TIMP1, MMP9/TIMP1 ratios; and the bone-remodeling biomarker RANKL (pg/mL). All clinical samples were coded and therefore blinded to the laboratory investigator (Dr Saloom). Excellent reliability and agreement of repeated measurements of GCF biomarker levels were demonstrated previously.[17]

Sample Size

Sample size calculation was based on a previous study investigating the levels of GCF biomarkers (MMP8, MMP9, interleukin-1β, osteoprotegerin, CRP) as predictors of periodontal disease progression.[25] In this investigation, differences in the levels of GCF biomarkers between participants with stable and progressing periodontitis were identified with a mean effect size of 2.77. Assuming a significance level of .05 and power of .80, a calculated sample size of eight subjects per group would be sufficient to detect a significant difference in GCF biomarker levels between the obese and normal-weight groups (G*Power 3.1.2). To allow for any dropouts, sample loss, or power underestimation between biomarkers, a total sample of 19 subjects in each experimental group was used.

Statistical Methods

Descriptive statistics were used to summarize outcome variables. Parametric and nonparametric analyses were carried out after checking for normality distribution using the Shapiro-Wilk test. Independent *t*-tests were used to compare normally distributed data (age, BMI, uWMS and GCF flow rate, plaque and gingival indices). The Mann-Whitney *U*-test was used to compare the nonnormally distributed biomarker concentration data. Pearson and Spearman correlation coefficients were used to measure the relationship between GCF biomarker concentration and plaque and gingival indices. All statistical analyses were done using IBM SPSS Statistics software (IBM Corporation, version 23, New York, NY).

RESULTS

Participants

This cohort study included 38 subjects (18 male, 20 female) with an overall mean age of 25.6 (6.3) years. Table 1 shows the essential demographics of the two cohorts. Mean BMI was 22.6 (1.6) in the normal-weight group and 32.4 (2.2) kg/m^2 in the obese group. Apart from BMI ($P < .0001$), there were no statistically significant differences in essential demographics between the two groups.

Table 2 shows the GCF biomarker levels in subjects within the study. There were significantly increased differences detected in the obese group compared with the normal-weight group for the obesity biomarker leptin and the tissue-remodeling biomarker MMP9. For the remainder of the tissue-remodeling biomarkers, the RANKL bone-remodeling marker, and the inflammatory markers, there were no significant differences between groups.

Some GCF biomarkers are produced as a response to local inflammation within the gingival tissues. Both normal-weight and obese subjects showed comparable plaque levels and gingival index scores with no significant differences between groups (see Table 1). No correlation was observed between plaque index or gingival index for any GCF biomarker for either group ($P = 1.00–.07$).

DISCUSSION

This cross-sectional study investigated a cohort of obese and normal-weight adult subjects during the final phase of fixed-appliance orthodontic treatment. Specifically, the biochemical profile of GCF samples derived from these subjects was investigated in an attempt to identify differences between these matched cohorts during fixed appliance orthodontic treatment. Significant differences were found in the levels of two GCF biomarkers between obese and normal-weight patients: the adipokine leptin and the tissue-remodeling enzyme MMP9.

The groups in this investigation were carefully matched and demonstrated equivalence in a range of baseline demographics including age, gender, uWMS and GCF flow rates, plaque and gingival indices. BMI represented the only significant demo-

Table 2. GCF Biomarker Levels of Subjects[a]

GCF Biomarker	Overall, Mean (SD)	Normal Weight, Mean (SD)	Obese, Mean (SD)	*P* Value
GCF flow rate, μL/min	0.92 (0.20)	0.86 (0.17)	0.89 (0.21)	.06[b]
Adiponectin, ng/mL	6481.07 (4439.60)	6683.80 (3224.31)	6278.34 (5480.16)	.258[c]
Leptin, pg/mL	353.03 (166.17)	298.30 (81.69)	407.77 (209.19)	**.009**[c]
Resistin, ng/mL	518.35 (278.51)	484.14 (280.57)	552.56 (279.73)	.435[c]
MPO, ng/mL	453.52 (302.87)	459.47 (319.76)	447.57 (293.67)	.773[c]
CRP, ng/mL	141.06 (286.95)	33.02 (121.61)	249.10 (360.31)	.075[c]
MMP8, ng/mL	3539.26 (943.77)	3310.32 (775.50)	3768.19 (1057.75)	.223[c]
MMP9, ng/mL	4452.52 (1434.44)	4117.81 (1209.17)	4787.23 (1590.90)	**.020**[c]
TIMP1, ng/mL	99.82 (67.62)	103.98 (76.43)	95.66 (59.34)	1.000[c]
MMP8/TIMP1	21.35 (12.70)	20.86 (14.00)	21.84 (10.86)	.603[c]
MMP9/TIMP1	15.54 (9.28)	15.10 (10.46)	15.99 (7.61)	.435[c]
RANKL, pg/mL	1192.24 (681.41)	1040.51 (324.95)	1343.97 (894.56)	.624[c]

[a] Significant results are indicated in bold. SD indicates standard deviation; GCF, gingival crevicular fluid; MPO, myeloperoxidase; CRP, C-reactive protein; MMP8, matrix metalloproteinase-8; MMP9, matrix metalloproteinase-9; TIMP1, tissue inhibitor of metalloproteinase 1; RANKL, receptor activator of nuclear factor kappa-B ligand.
[b] Independent *t*-test.
[c] Mann-Whitney *U*-test.

graphic difference between the two cohorts. However, significant differences were identified in two GCF parameters, which included increased levels of the proinflammatory adipokine leptin and the tissue-remodeling MMP9. Collectively, these findings provided some evidence of an increased inflammatory status within the periodontium of obese individuals in the later stages of orthodontic treatment with fixed appliances when compared with normal-weight subjects. However, not all of the biomarkers assayed were significantly different between groups, including the additional adipokines adiponectin and resistin, a selection of inflammatory mediators, and both tissue- and bone-remodeling biomarkers. This was possibly a reflection of the complex biochemistry that underlies a systemic condition such as increased BMI and also the processes that regulate orthodontic tooth movement.[11]

A relationship was previously shown between the levels of several GCF biomarkers and rate of orthodontic tooth movement during alignment with fixed appliances in obese subjects when compared with normal-weight subjects, and among these biomarkers, leptin was significantly increased in the obese group.[17] As a mediator of chronic inflammation, this invited speculation that a baseline proinflammatory state in the periodontium of obese subjects might facilitate an increased early response of the dentition to orthodontic force. Leptin has also been studied in relation to orthodontic tooth movement in two other investigations.[26,27] Salivary leptin levels were assayed in normal-weight and overweight female subjects undergoing canine retraction with fixed appliances. In this investigation, mean salivary leptin concentration was also significantly greater in the overweight subjects at all time points. Leptin levels were significantly increased in both groups at 1 hour following force application but fell to baseline levels by 1 month.[27] Although this study did not address leptin concentrations in GCF, it was consistent with increased GCF leptin in subjects classified as obese.[17,27] However, the biochemistry is likely to be complex, and normal-weight adolescent subjects have been shown to have a GCF leptin concentration that actually decreases in a time-dependent manner during canine retraction with fixed appliances, with these changes reaching significance by 168 hours.[26] MMP9 is a gelatinase associated with the degradation of denatured collagen during soft tissue remodeling and is upregulated in the periodontium in association with orthodontic tooth movement.[12,28] Interestingly, increased GCF MMP9 levels have previously been found in association with healthy volunteers undergoing canine retraction with fixed appliances,[12] although no differences in obese subjects compared with normal-weight subjects have been described.[17] Overall, there have been a number of clinical studies in orthodontics describing assays for various biochemical mediators of orthodontic tooth movement in GCF. The results of these studies have varied, which almost certainly reflects the relatively heterogeneous subject cohorts and stages of treatment that have been investigated.[8,29]

The strengths of the present study included adequate power of the sample size, baseline comparability between experimental groups including measured assays of gingival plaque and inflammation, an absence of dropouts, the use of blinding in the laboratory, and a sample size based on a robust power calculation. In addition, obesity was defined and classified according to recommended and accepted

international standards. However, this was a cross-sectional study that evaluated clinical parameters between groups at only a single time point. In addition, while all subjects had been in 0.019 × 0.025-inch stainless-steel wires for at least 1 month, some heterogeneity in treatment mechanics was inevitable, and this might have influenced the results.

CONCLUSION

- This cross-sectional clinical cohort study investigated obese and normal-weight subjects undergoing fixed-appliance orthodontic treatment. The biochemical profile of GCF samples derived from the lower incisor region of these subjects following alignment showed significantly increased levels of the adipokine leptin and the tissue-remodeling biomarker MMP9. These data provide further evidence of biochemical differences in the GCF of obese and normal-weight subjects undergoing orthodontic treatment with fixed appliances.

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REFERENCES

1. Ng M, Fleming T, Robinson M, et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet*. 2014;384:766–781.
2. Deng T, Lyon CJ, Bergin S, Caligiuri MA, Hsueh WA. Obesity, inflammation, and cancer. *Ann Rev Path*. 2016;11:421–449.
3. Hotamisligil GS. Inflammation and metabolic disorders. *Nature*. 2006;444:860–867.
4. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol*. 2011;11:85–97.
5. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature*. 1994;372:425–432.
6. Steppan CM, Bailey ST, Bhat S, et al. The hormone resistin links obesity to diabetes. *Nature*. 2001;409:307–312.
7. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem*. 1995;270:26746–26749.
8. Kapoor P, Kharbanda OP, Monga N, Miglani R, Kapila S. Effect of orthodontic forces on cytokine and receptor levels in gingival crevicular fluid: a systematic review. *Prog Orthod*. 2014;15:65.
9. Marcaccini AM, Amato PA, Leao FV, Gerlach RF, Ferreira JT. Myeloperoxidase activity is increased in gingival crevicular fluid and whole saliva after fixed orthodontic appliance activation. *Am J Orthod Dentofacial Orthop*. 2010;138:613–616.
10. Navarro-Palacios A, Garcia-Lopez E, Meza-Rios A, Armendariz-Borunda J, Sandoval-Rodriguez A. Myeloperoxidase enzymatic activity is increased in patients with different levels of dental crowding after initial orthodontic activation. *Am J Orthod Dentofacial Orthop*. 2014;146:92–97.
11. MacLaine JK, Rabie AB, Wong R. Does orthodontic tooth movement cause an elevation in systemic inflammatory markers? *Eur J Orthod*. 2010;32:435–440.
12. Grant M, Wilson J, Rock P, Chapple I. Induction of cytokines, MMP9, TIMPs, RANKL and OPG during orthodontic tooth movement. *Eur J Orthod*. 2013;35:644–651.
13. Papageorgiou SN, Reichert C, Jager A, Deschner J. Effect of overweight/obesity on response to periodontal treatment: systematic review and a meta-analysis. *J Clin Periodontol*. 2015;42:247–261.
14. Keller A, Rohde JF, Raymond K, Heitmann BL. Association between periodontal disease and overweight and obesity: a systematic review. *J Periodontol*. 2015;86:766–776.
15. Suvan J, D'Aiuto F, Moles DR, Petrie A, Donos N. Association between overweight/obesity and periodontitis in adults: a systematic review. *Obes Rev*. 2011;12:e381–e404.
16. Suvan J, Petrie A, Moles DR, et al. Body mass index as a predictive factor of periodontal therapy outcomes. *J Dent Res*. 2014;93:49–54.
17. Saloom HF, Papageorgiou SN, Carpenter GH, Cobourne MT. Impact of obesity on orthodontic tooth movement in adolescents: a prospective clinical cohort study. *J Dent Res*. 2017;96:547–554.
18. Neeley WW II, Dolce C, Hatch JP, Van Sickels JE, Rugh JD. Relationship of body mass index to stability of mandibular advancement surgery with rigid fixation. *Am J Orthod Dentofacial Orthop*. 2009;136:175–184.
19. Neeley WW II, Gonzales DA. Obesity in adolescence: implications in orthodontic treatment. *Am J Orthod Dentofacial Orthop*. 2007;131:581–588.
20. von Bremen J, Lorenz N, Ruf S. Impact of body mass index on oral health during orthodontic treatment: an explorative pilot study. *Eur J Orthod*. 2016;38:386–392.
21. von Bremen J, Lorenz N, Ludwig B, Ruf S. Increased BMI in children-an indicator for less compliance during orthodontic treatment with removable appliances. *Eur J Orthod*. 2018;40:350–355.
22. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J Clin Epidemiol*. 2008;61:344–349.
23. Loe H, Silness J. Periodontal disease in pregnancy. I. Prevalence and severity. *Acta Odontol Scand*. 1963;21:533–551.
24. Silness J, Loe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condtion. *Acta Odontol Scand*. 1964;22:121–135.
25. Kinney JS, Morelli T, Oh M, et al. Crevicular fluid biomarkers and periodontal disease progression. *J Clin Periodontol*. 2014;41:113–120.

26. Dilsiz A, Kilic N, Aydin T, Ates FN, Zihni M, Bulut C. Leptin levels in gingival crevicular fluid during orthodontic tooth movement. *Angle Orthod*. 2010;80:504–508.
27. Jayachandran T, Srinivasan B, Padmanabhan S. Salivary leptin levels in normal weight and overweight individuals and their correlation with orthodontic tooth movement. *Angle Orthod*. 2017;87:739–744.
28. Takahashi I, Onodera K, Nishimura M, Mitnai H, Sasano Y, Mitani H. Expression of genes for gelatinases and tissue inhibitors of metalloproteinases in periodontal tissues during orthodontic tooth movement. *J Mol Histol*. 2006;37:333–342.
29. Alhadlaq AM. Biomarkers of orthodontic tooth movement in gingival crevicular fluid: a systematic review. *J Contemp Dent Pract*. 2015;16:578–587.