

Effect of ten different biomarkers in the gingival crevicular fluid of obese and non-obese undergoing fixed orthodontic treatment

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Abstract. – OBJECTIVE: The aim of this study is to evaluate the effect of ten proinflammatory cytokines in GCF of participants with raised body mass index (BMI) compared to non-obese subjects undergoing fixed orthodontic treatment.

PATIENTS AND METHODS: In the cross-sectional cohort, subjects were shortlisted through the purposive sampling method with the same age and gender and similar characteristics (cohort). For inclusion and exclusion, predefined criteria were followed. In all included participants obese and non-obese collection of GCF was made from mandibular canine to canine. Identification of inflammatory mediators (MPO and CRP) leptin, adiponectin, and resistin (pg/mL). Bone remodeling biomarkers RANKL (pg/mL) and tissue remodeling biomarkers MMP8, MMP9, TIMP1, and MMP8/TIMP1, MMP9/TIMP1 ratio were collected and blinded by the investigator. Normal distribution of data i.e., age, BMI, the flow rate of GCF, indices plaque and gingival, and uWMS were compared using a *t*-test. Non-normality biomarker data were evaluated using Mann-Whitney U-test. To assess the relationship between the concentration of GCF biomarkers and plaque and gingival indices Pearson and Spearman correlation coefficients were used.

RESULTS: The total number of participants included was 44. In the obese and non-obese groups, the male/female ratio was the same i.e., (n=11 each). The mean age of participants in the obese group was 25.7±1.55 years, whereas the non-obese group was 26.1±1.29 years. In obese the mean BMI was 33.6±2.1 kg/m² whereas in non-obese 22.9±1.9 kg/m² (*p*<0.02). Among the levels of biomarkers adiponectin (*p*<0.006) and leptin (*p*<0.028) demonstrated a significant dif-

ference between obese and non-obese participants. Also, a significant difference was noted between obese and non-obese in tissue remodeling biomarker MMP9 (*p*<0.03).

CONCLUSIONS: A surge in the level of the biomarkers, i.e., MMP9, leptin, and adiponectin in the gingival crevicular fluid is found in obese undergoing fixed orthodontic treatment.

Key Words:

Gingival crevicular fluid, Obese, Non-obese, Fixed orthodontic appliance, Biomarkers.

Introduction

Urbanization has augmented the incidence of obesity in developed countries over the past few decades¹. The uprise in the prevalence of the disease has burdened the healthcare system due to the already established relationship between obesity with stroke, cardiovascular disease (CVD), diabetes, hypertension, and cancer². Obesity is the excessive accumulation of adipose tissue along with a chronic inflammatory state³. The principal cellular constituent of adipose tissues are adipocytes that produce adipokines which discursively impact inflammation and various systemic metabolic function⁴. These adipokines may range from resistin, leptin, and adiponectin which is anti-inflammatory^{5,6}.

The orthodontic tooth movement occurs through the remodeling of the connective tissue with a localized inflammatory response to chang-

es in alveolar bone and periodontium due to induction of external force^{7,8}. During the process, a variety of biochemical mediators are produced, many of these mediators are found in the gingival crevicular fluid (GCF). These inflammatory mediators may include bone-remodeling biomarker receptor activator of nuclear factor kappa-B ligand (RANKL), inflammatory biomarkers C-reactive proteins (CRP), and myeloperoxidase (MPO). Remodeling biomarkers may include matrix metalloproteinase-8 (MMP8), matrix metalloproteinase-9 (MMP9), and tissue inhibitor biomarkers metalloproteinase 1 (TIMP1)⁹⁻¹¹. As evidence advocates, raised body mass index (BMI) is correlated with chronic systemic inflammation, hence periodontal health, and different periodontal parameters i.e., bleeding on probing (BoP), probing depth (PD), and plaque index (PI) of obese participants are compromised with delayed healing and recurrent periodontal infection with poor response to periodontal therapy¹²⁻¹⁴.

Orthodontic tooth movement is already investigated in obese participants, based on the narrative that periodontal proinflammatory changes in periodontal tissues of overweight individuals influence the rate of tooth movement^{15,16}. Saloom et al¹⁷ found in their recent work that after adjustment of confounders in a given cohort, overweight participants had a higher rate of tooth movement compared to nonobese. Further inferences of obesity for orthodontic treatment have also been debated which may vary from growth pattern (both pubertal and craniofacial), to treatment stability and psychosocial well-being. Obese participants with fixed and removable orthodontics also behave as a risk factor for compromised /poor oral health, increasing the time of treatment duration; lacking of cooperation^{18,19}.

The present study is formulated on the hypothesis that cytokine profiles in GCF in obese and non-obese participants undergoing orthodontics will be indifferent. Therefore, the present cohort study aimed to evaluate the effect of ten proinflammatory cytokines in the GCF of participants with raised BMI compared to non-obese subjects undergoing fixed orthodontic treatment.

Patients and Methods

The current cross-sectional cohort study was in harmony with STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines. The ethical approval was tak-

en from Prince Sattam Bin Abdulaziz University along with the written consent acknowledged by all participants. The duration of the study was six months. The recruitment of participants was carried out by the outpatient department of Orthodontics, Dar Al Uloom University.

The subjects were shortlisted through the purposive sampling method with the same age and gender and similar characteristics (cohort). The predefined criteria were as follow: age of participants 18-45 years, need for fixed orthodontic treatment 0.022-inch orthodontic bracket (Koden, Germany), prescription MBT, and stainless steel archwire 0.020×0.025 for 1 month (Zhejiang Shinye Medical Technology Corp., Ltd.), patients having no medical issue or use of antibiotics or analgesics in the last six months, and non-smokers. Participants having a BMI of (18.5-24.9) were considered non-obese and obesity was classified as BMI ≥30. Using a calibrated scale the nearest 0.1 kg body weight was measured. Wall mounted ruler was used to measuring the height to the nearest centimeter. The calculation of BMI was performed based on the following formula kg/m^2 . A single experienced trained dental nurse took all the measurements to minimize bias.

GCF Sample Collection

Sample collection was performed during a routine orthodontic appointment during orthodontic timings (10 am to 3 pm) after one month of placement of stainless steel archwire 0.020×0.025. The unstimulated salivary flow rate (USFR) was measured as saliva drooling in millimeters collected from a relaxed patient in a test tube in 300 seconds. The health of the periodontium was measured using both gingival and plaque index.

The collection of GCF was made from mandibular canine to canine from the distal side involving six teeth. Initially, teeth were dried and isolated with an air syringe. White filter paper strips (Periopaper, flow-ora, NY, USA) were positioned at 1 mm distally in the gingival crevice for 30 sec). Contaminated strips with blood and saliva were discarded. The volume of GCF on the collected strip was measured using a Periotron (Flow-ora, NY, USA). Readings were converted to volume by reference to the average curve and flow rate measured per 60 sec. From the filter strips, GCF was retrieved in the presence of phosphate-based buffered solution 20IL, and plates were coated with specific protein antibodies and centrifuged by 300 sec. Beaded-based

multiplex assay by Luminex magnetic kit for detection available commercially (R&D systems, Abingdon, UK) was used for the identification of inflammatory mediators (MPO and CRP) leptin adipokines and adiponectin and resistin (pg/mL). Bone remodeling biomarkers RANKL (pg/mL) and tissue remodeling biomarkers MMP8, MMP9, TIMP1, and MMP8/TIMP1, MMP9/TIMP1 ratio. All clinically collected samples were coded and blinded by the investigators. Kappa score intra-examiner reliability was measured which was calculated as 0.88.

Calculation of Sample Size

For sample size calculation, 11 participants were found to be enough in each group, assuming a level of significance of 0.05 and power of the study of 0.80 to perceive a significant difference in the levels of biomarkers in GCF. However, since the underestimation of power, the sample loss, and the drop in follow-up were considered. A sample of 22 subjects in each group was calculated to be enough i.e., 44 subjects in both experimental groups.

Statistical Analysis

Different outcome variables were summarized using descriptive statistics. The normality of the data was assessed using the Kolmogorov-Smirnov test followed by para- and nonparametric tests. Normal distribution of data i.e., age, BMI, the flow rate of GCF, indices plaque and gingival, and uWMS were compared using a *t*-test. Non-normality biomarker data were evaluated using Mann-Whitney U-test. To assess the relationship between the concentration of GCF biomarkers and plaque and gingival indices Pear-

son and Spearman correlation coefficients were used. The software program for social sciences (SPSS Version 24, Armonk, NY, USA) was used for statistical analysis with a significance threshold of *p*<0.05.

Results

In the present cohort study, the total number of participants included was 44. In the obese and non-obese groups, the male/female ratio was the same i.e., (n=11 each). The mean age of participants in the obese group was (25.7±1.55 years). Whereas, the non-obese group was (26.1±1.29 years). In obese the mean BMI was (33.6±2.1 kg/m²) whereas in non-obese (22.9±1.9 kg/m²) (*p*<0.02). There was no significant difference between UWMS (*p*>0.69), gingival (*p*>0.71), and plaque index (*p*>0.33) among obese and non-obese participants (Table I).

Table II demonstrates levels of biomarkers in GCF in obese and non-obese participants. Among the participants, adiponectin (*p*<0.006) and leptin (*p*<0.028) demonstrated a significant difference between obese and non-obese participants. Also, a significant difference was noted between obese and non-obese in tissue remodeling biomarker MMP9 (*p*<0.03). Among others, inflammatory biomarkers (CRP, MPO), bone remodeling biomarkers (RANKL), tissue inhibitor biomarkers (TIMP1), and remaining tissue remodeling biomarkers (MMP8) no significant difference was noted (*p*>0.05). Between biomarkers in GCF, plaque index, and gingival index, no correlation was observed among participants in both groups.

Table I. Basic demographic characteristics of participants.

Demographics	Obese	Non-obese	<i>p</i> -value
Number of Participants	22	22	-
Age (years)	25.7±1.55	26.1±1.29	0.56 ⁱ
Male/female	11/11	11/11	-
BMI kg/m ²	33.6±2.1	22.9±1.9	0.02ⁱ
UWMS mL//min	0.63±0.22	0.69±0.14	0.69 ⁱ
Gingival index	2.33±0.21	1.99±0.17	0.71 ⁱ
Plaque Index	1.39±0.13	1.22±0.10	0.33 ⁱ

Demographics are given in mean and SD The *p*-value in bold and italics shows the level of significance BMI: Body mass index; UWMS: unstimulated whole mouth salivary rate ! Independent *t*-test.

Table II. Level of biomarkers in GCF in obese and non-obese participants.

Biomarker in GCF	Obese (Mean±SD)	Non-obese (Mean±SD)	p-value
GCF flow rate, IL/min	0.79±0.21	0.76±0.13	0.98 [†]
Adiponectin, ng/mL	5981.54±326	6958.17±458	0.006^Ω
Leptin, pg/mL	501.25±32.21	235.21±17.85	0.028^Ω
Resistin, ng/mL	521±15.25	487±17.31	0.66 ^Ω
MPO, ng/mL	471.54±87.25	487.88±91.33	0.34 ^Ω
CRP, ng/mL	31.25±111.27	247.21±354.87	0.74 ^Ω
MMP8, ng/mL	3247.85±356.98	3547.02±485.54	0.27 ^Ω
MMP9, ng/mL	5236.4±723.1	4236.2±614.3	0.03^Ω
TIMP1, ng/mL	107.84±51.3	150±49.9	0.47 ^Ω
MMP8/TIMP1	25.36±5.37	24.36±4.21	0.78 ^Ω
MMP9/TIMP1	17.25±3.55	17.65±3.47	0.69 ^Ω
RANKL, pg/mL	1548.74±765.21	1174±569.32	0.11 ^Ω

The p-value in bold and italics shows the level of significance [†]Independent *t*-test; ^ΩMann-Whitney U-test.

Discussion

The present study expressed the hypothesis that cytokine profiles in GCF in obese and non-obese participants undergoing orthodontics will be indifferent. There was partial acceptance of the hypothesis as some cytokines in GCF i.e., adiponectin, leptin, and tissue-remodeling enzyme MMP9 demonstrated a significant difference in obese participants compared to non-obese participants undergoing fixed orthodontics during the final phases of the treatment. The difference in three biomarkers in GCF speculates an increase in inflammation of periodontal tissues in obese participants. While other biomarkers remain indifferent. This finding is a reflection of complex biochemistry that underlies systemic condition like obesity with increased BMI and the procedure that control tooth movement. Baseline demographics in each group were matched and homogenized i.e., age, gender, BMI, uWMS, and flow rate of GCF. The only demographic significant difference between obese and non-obese subjects in a given cohort was BMI.

Leptin is produced by adipocytes and is a polypeptide hormone^{20,21}. Initially, its presence was found to be related to a person with an increased BMI²². Later, it was noted that leptin has a dual role as a cytokine and hormone²³. BMI has a strong correlation with leptin levels in obese^{24,25}. Evidence suggests that leptin levels increase the metabolism of bone in obese participants undergoing fixed orthodontic treatment which may influence orthodon-

tic tooth movement^{22,26}. It is estimated that leptin has both effects on tooth movement i.e., inhibitory and stimulatory^{27,28}. The rate of tooth movement was not assessed in the present study, which was the constraint of the present study. Recent work by Jayachandran²⁶ showed that leptin concentration varied at different times in saliva. The present study assessed leptin levels in GCF during the final phases of the treatment. Therefore, it will be wise to assume that leptin levels fluctuate in saliva but remains constant in GCF. This was also the finding of the present study that in GCF leptin levels were found to be high with increased BMI.

Another, biomarker in GCF that was found to be significantly higher in obese participants undergoing fixed orthodontic movement was adiponectin. Adiponectin plays a major role in periodontal and bone remodeling^{29,30}. The latest works^{29,31} suggests that the concentration of adiponectin decrease in obese and soon increases after the loss of weight. Moreover, in rats, a low concentration of adiponectin is related to an increase in collagen content in the periodontal region and results in a significant decrease in tooth mobilization²⁹. The authors of the present study believe that a low concentration of adiponectin is beneficial for tooth movement. Since tooth movement was not measured in the initial and final stages of orthodontic treatment, this remains an inherent limitation of the present study. More studies are validated to measure tooth movements with levels of leptin and adiponectin for better and conclusive outcomes^{29,30}.

Tissue-remodeling enzyme MMP9 is allied to the degradation of denatured collagen during the remodeling of soft tissue while they are regulated by tissue inhibitor metalloproteinases (TIMPs)³². Also, the biomarker is related to tooth movement in patients with orthodontic treatment, inflammation of periodontium, and bone degradation^{33,34}. MMPs are involved in the degradation of the extra-cellular matrix during the remodeling phase³³. In the present study, a hike in MMP9 biomarkers was related to orthodontic treatment and periodontal inflammation in obese³⁵⁻³⁷. All the factors contributed to its upsurge. This association is already documented and reported by Sioustis et al³⁶ and Bildt et al³⁷. To our knowledge, there had been multiple studies^{10,38,39} on the levels of biomarkers/cytokines in patients undergoing orthodontic treatment with varied results. The disparity in results is due to heterogeneity in a cohort, stage of treatment, and type of orthodontic appliance (aligners and fixed orthodontics)⁴⁰⁻⁴³.

Strengths and Limitations

Some advantages of the present study include blinding to minimize bias, adequate power of the study, baseline measurements, investigation of periodontal parameters, no loss to follow-up of the participants, and adequate sample size in each group. Limitations of the current study as mentioned previously are the non-measuring of tooth movement; non-investigation of radiographic parameters i.e., crestal bone loss, and not gauging qualitative parameters in obese and non-obese participants undergoing fixed orthodontic treatment.

Conclusions

A surge in the level of the biomarkers, i.e., MMP9, leptin, and adiponectin in the gingival crevicular fluid is found in obese undergoing fixed orthodontic treatment. Further, investigations and clinical trials are encouraged to extrapolate the verdicts of the current study.

Conflict of Interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Ethics Approval

The ethical committee of Prince Sattam bin Abdulaziz University approved the study.

Informed Consent

Written consent was accepted by all participants.

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Authors' Contribution

Conceptualization, F.A, A.M. and S.N.A.; Methodology, A.A., and S.N.A.; Software, A.A. and S.A.; Validation, S.N.A., A.A., and A.A.; A.S Formal analysis, A.S; A.R; Investigation, A.A; S.A Resources, N.A.; data curation, A.A; A.R, A.S writing-original draft preparation, S.A., and A.S.; writing—review and editing, A.S.; visualization, A.S; supervision, F.A, A.A, A.S, and A.M. and A.R.; project administration, A.A.; funding acquisition, A.A. All authors have read and agreed to the published version of the manuscript.

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