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ORIGINAL ARTICLE

Cytokine levels in gingival crevicular fluid during orthodontic treatment with aligners compared to conventional labial fixed appliances: a 3-week clinical study

Anadha N. Gujar[a], Hosam Ali Baeshen[b], Anwar Alhazmi[c], Shilpa Bhandi[c], A. Thirumal Raj[d], Shankargouda Patil[c] and Dowen Birkhed[e]

[a]KLES, Institute of Dental Sciences, Bangalore, India; [b]College of Dentistry, King Abdulaziz University and Alfarabi Private College for Dentistry and Nursing, Jeddah, Saudi Arabia; [c]College of Dentistry Jazan University, Jazan, Saudi Arabia; [d]Sri Venkateswara Dental College and Hospital, Chennai, India; [e]Professor Emeritus at the University of Gothenburg, Gothenburg, Sweden

ABSTRACT

Objective: To test the hypothesis that the levels of IL-1ß and TNF-α increased more and IL-1α, IL-2, IL-6, IL-8 increased less, after 3 weeks of treatment with conventional labial fixed appliance and with aligners.

Material and methods: Forty patients who were treated either with labial brackets ($n = 20$) or aligners ($n = 20$). Gingival crevicular fluid (GCF) samples were collected at baseline and after 21 days. Cytokine levels were evaluated by enzyme-linked immune sorbent assay (ELISA). Plaque index (PI), gingival index (GI), and bleeding on probing (POB) were also examined.

Results: The levels of IL-1α, IL-1ß, IL-2, IL-6, IL-8 and TNF-α in the GCF were significantly increased in both groups. The levels of IL-2, IL-6, IL-8 increased more in patients treated with aligners compared to those treated by labial fixed appliances. There was a statistically significant difference in change of the mean cytokine levels of IL-1α, IL-2, IL-6, IL-8 and TNF-α compared to labial fixed appliances and aligners.

Conclusions: The levels of the six studied cytokines in GCF (IL-1α, IL-1ß, IL-2, IL-6, IL-8 and TNF-α) increased after 3 weeks both after treatment with conventional labial fixed appliance and with aligners. IL-1ß and TNF-α showed a prominent increase compared to the other cytokines in the GCF of teeth by both the labial fixed appliance and aligners. However, there were only minor differences in the changes of the cytokine levels from baseline to 3 weeks between the two groups. There were no differences between the groups regarding PI, GI or POB.

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Aligners; bone biology; cell/molecular biology; labial fixed appliances

Introduction

Orthodontic appliances apply forces onto the periodontal ligament tissues, which in turn leads to modelling and remodelling of the alveolar bone [1]. The mechanical stresses due to the orthodontic forces stimulate the periodontal ligament cells to produce biologically active substances that cause remodelling of connective tissue and activation of osteoclasts [2].

Gingival crevicular fluid is the sulcular fluid and is considered an inflammatory exudate; its formation was first defined by Alfano [3]. Proteins, cytokines, bacterial antigens, electrolytes, small organic molecules and enzymes of both host and bacterial origin are the molecules isolated from the sulcular fluid [4,5].

Inflammation in the periodontal tissues is seen due to orthodontic forces applied for tooth movement and due to the accumulation of dental plaque [1,6]. This inflammatory response is associated with the release of a variety of cytokines, matrix metalloproteinases, osteoprotegerin, lactate dehydrogenase, tumour necrosis factor (TNF), etc. in GCF [6,7]. Cytokines are biomarkers that are considered potent stimulators of bone resorption [8].

Aligners are currently in use due to the aesthetic demands of society, and despite their spread among the orthodontic community; there are not many studies describing the bone metabolism induced by them [1]. The design of these appliances is one of the important factors in plaque accumulation due to their different retentive areas [2]. Kesling introduced the use of clear thermoplastic appliances for aligning the teeth in 1946, but only after Align technology (Santa Clara, CA, USA) launched the Invisalign system in 1998, these appliances got prescribed on a large scale with the introduction of CAD/CAM to orthodontics [9]. Invisalign make use of series of transparent aligners made up of polymer material for moving the teeth in planned position in 'virtual treatment' using a software programme [10]. Compared to fixed appliances, removable appliances like aligners facilitate the maintenance of oral hygiene [11–13]. The main component of aligners is clear plastic splints covering all the teeth plus the marginal aspects of the gingiva, which gradually move the teeth into an ideal position. The system combines characteristics of

CONTACT Dowen Birkhed birkhed@gmail.com Fersens väg 14B, SE-211 42 Malmö, Sweden

fixed and of removable appliances [14]. Apart from the numerous other advantages of aligners, the main advantage is the aesthetics and comfort of the patient. However, clear aligners have some disadvantages, including higher costs and the inability to treat certain types of malocclusion [15].

Very limited literature is available on the production of mediators during orthodontic tooth movement in humans. No study has compared the production of various cytokines in patients treated with a labial fixed appliance and aligners. Therefore, the present study was undertaken to evaluate and compare the level of cytokines in patients treated with a labial fixed appliance and in patients treated with aligners.

Materials and methods

The orthodontic treatment affects the dental health such as improving the functional problems (mastication, deglutition, respiration, etc), management of temporomandibular joint functions, prevention of trauma from occlusion, management of speech and psychological problems, which would affect the social life of the individual [16,17]. Correction of malocclusion with orthodontic treatment leads to an improvement in oral health-related quality of life [17]. Elimination of functional problems that may cause temporomandibular dysfunction, improving facial and dental aesthetics by alignment and levelling, encouraging eruption of impacted teeth, establishing mutually protective occlusion by correcting the overjet and overbite, etc. are the main indications of orthodontic treatment [16–20].

Patients and study design

Participants who were undergoing orthodontic treatment with a metallic labial fixed appliance and aligners were recruited from the Department of orthodontics and dentofacial orthopaedics at KLE Society's institution of dental sciences, Bangalore, India. The Institutional Ethics Committee (IEC) at KLE Society's Institute of dental sciences, Bangalore, approved the study (IEC No. KIDS/IEC/APR 18-4). The participants received both verbal and written information about the study and signed consent forms were obtained before starting the study. To avoid treatment bias, only patients diagnosed with the same clinical presentation (mild malocclusion between 2.1 and 4.0 mm) [21,22] were included in the study. To avoid selection bias all the included cases were randomly allocated into two treatment groups. The sample consisted of forty patients (age 12–32 years; mean 28 ± 4 years), of whom twenty patients were undergoing treatment with a labial fixed appliance (12 females, 8 males) and twenty patients were undergoing treatment with aligners (11 females, 9 males).

The cases included in our study exhibited healthy systemic conditions, required orthodontic treatment, and showed no use of anti-inflammatory drugs in the month preceding the study, no use of antibiotic drugs in the past 6 months, periodontal health with GI score <1, no pockets with generalized probing depths ≤ 3 mm and no radiographic evidence of periodontal bone loss after a full-mouth radiographic periapical examination. Patients with smoking habits, signs of gingivitis or periodontitis or a diagnosis of systemic disease were excluded from our study. Eligible participants were selected from patients of both sexes with complete dentitions. Informed consents were obtained from the patients and the parents of patients under 18 years of age. During the month preceding the preliminary examination, all the patients received repeated oral hygiene instructions about the correct use of a toothbrush, toothpaste, dental floss and interdental brush. The patients with fixed orthodontic appliances were advised to use orthodontic brushes (STIM) twice daily while those using aligners were advised to use regular toothbrush twice daily. The patients in both groups were advised to use Amflor fluoridated mouthwash (Group Pharmaceuticals) once daily. Two weeks before beginning the study, all the patients underwent supragingival prophylaxis. Moreover, the study subjects were not allowed to take any anti-inflammatory drugs during the study as that might affect the results [23]. Motivation was maintained during the entire study.

Periodontal examination

At the first visit (baseline), the Silness and Løe Plaque (PI) Index, Lobene Modified Gingival Index (GI), and Bleeding on Probing (BOP) Index were recorded. The six index teeth for assessment of plaque index were maxillary right first molar, maxillary right lateral incisor, maxillary left first bicuspid, mandibular left first bicuspid, mandibular left first molar, mandibular left lateral incisor and mandibular right first bicuspid. The four gingival areas of the tooth were examined distofacial, facial, mesiofacial and lingual surfaces. For obtaining the modified gingival index, the labial and lingual surfaces of gingival margins and interdental papilla of all erupted teeth except third molars were examined and scored. The same was recorded at the follow-up (Table 1).

GCF sampling

GCF samples were collected at baseline, i.e. before the start of orthodontic treatment (T0), and after 21 days from the start of orthodontic treatment (T1). The samples at T0 formed the baseline and those collected at T1 formed the follow-up. GCF samples were collected after 21 days from the start of orthodontic treatment because indirect resorption processes start on day 21 [24–29]. The site where the

Table 1. Comparison of mean cytokine values between labial fixed appliance ($n = 20$) and aligners ($n = 20$) groups using unpaired *t*-test.

Factors	Groups	*N*	Mean	SD	Mean Diff	*t* value	*p* Value
TNF-α	LABIAL FA	20	113.56	17.58	61.71	14.03	.000
	ALIGNERS	20	51.85	8.80			
IL8	LABIAL FA	20	0.77	.053	−0.39	−17.48	.000
	ALIGNERS	20	1.175	0.086			
IL6	LABIAL FA	20	1.13	0.108	−0.059	−14.74	.000
	ALIGNERS	20	1.73	0.145			
IL2	LABIAL FA	20	2.78	0.261	−1.34	−10.80	.000
	ALIGNERS	20	4.130	0.49			
IL-1α	LABIAL FA	20	0.520	0.246	0.458	8.320	.000
	ALIGNERS	20	0.0627	0.005			
IL-1β	LABIAL FA	20	7.92	7.25	3.33	2.054	.057
	ALIGNERS	20	4.58	0.179			

gingival inflammation was minimal/absent was selected. Selection of sample site was done according to convenience. The sample site was standardized for all subjects to the proximal region of either of the maxillary canines [6,30,31]. Isolation of the area was performed with the help of sterile gauze to prevent contamination from saliva. Using a microcapillary pipette (Sigma-Aldrich, Inc), GCF was collected by placing the pipette at the entrance of the gingival sulcus and gently touching the gingival margin [6,30–32]. An attempt was made to collect a standardized volume of 1 µl using the calibration on the white colour-coded 0.2–2 µL calibrated volumetric microcapillary pipette with an extracrevicular approach from each test site. The micropipettes that were contaminated by blood or saliva were excluded from the study. The GCF collected was immediately transferred to Eppendorf tubes (0.5 ml), centrifuged at 3000 rpm for 10 minutes and stored at −80 °C until the time of assay [33]. The patients were coded to avoid their direct identification. The data analyst was also blinded from their identification.

Analysis of cytokine molecules in GCF using enzyme-linked immuno-sorbent assay (ELISA)

The frozen GCF samples were thawed. Commercial test systems (Raybiotech, Inc. Norcross-GA, USA) were used for performing the ELISA analyses. In all, 240 samples were collected from patients who had been treated with a labial fixed appliance, and 240 samples were collected from patients who had been treated with aligners, resulting in a total of 480 samples. Of the 240 samples from each group, 40 samples (20 cases, 20 controls) were collected to evaluate a single cytokine molecule. Therefore, for evaluating six cytokine molecules, 240 samples were collected from a single appliance type.

Data analysis

Statistical Package for Social Sciences [SPSS] for Windows, Version 22.0 (Released 2013). Armonk, NY: IBM Corp, was used to perform statistical analyses. Mean of IL-1α, IL-1ß, IL-2, IL-6, IL-8 and TNF-α levels were calculated for each group. Paired *t*-test was used to compare the mean values of different biomarker levels [in pg/ml] between baseline and follow-up in both the groups studied. Comparison of mean cytokine values between the labial fixed appliance and aligners groups was using unpaired *t*-test. The level of significance was set at $p < .05$.

Results

Plaque index, gingival index and bleeding on probing in each group

There was no statistically significant difference in each index when compared at the baseline and at the follow-up in both the groups (Figure 1).

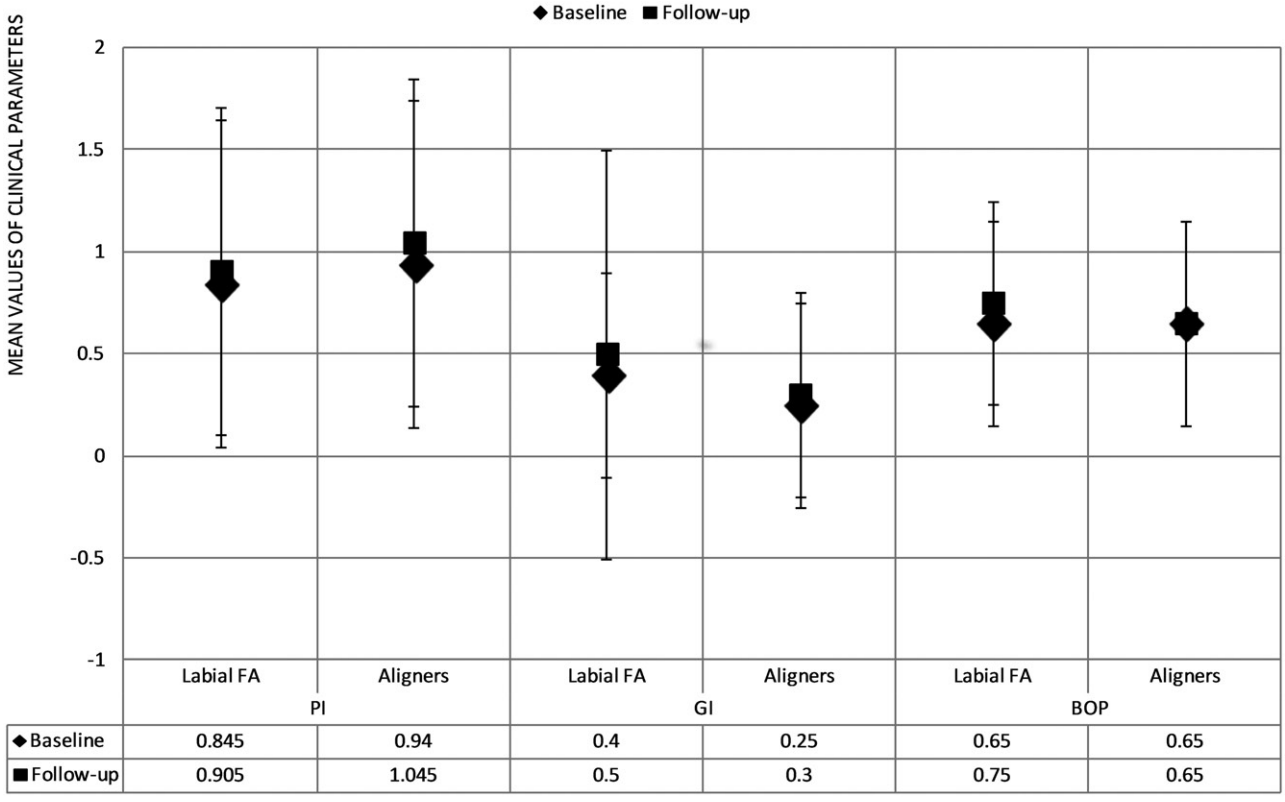


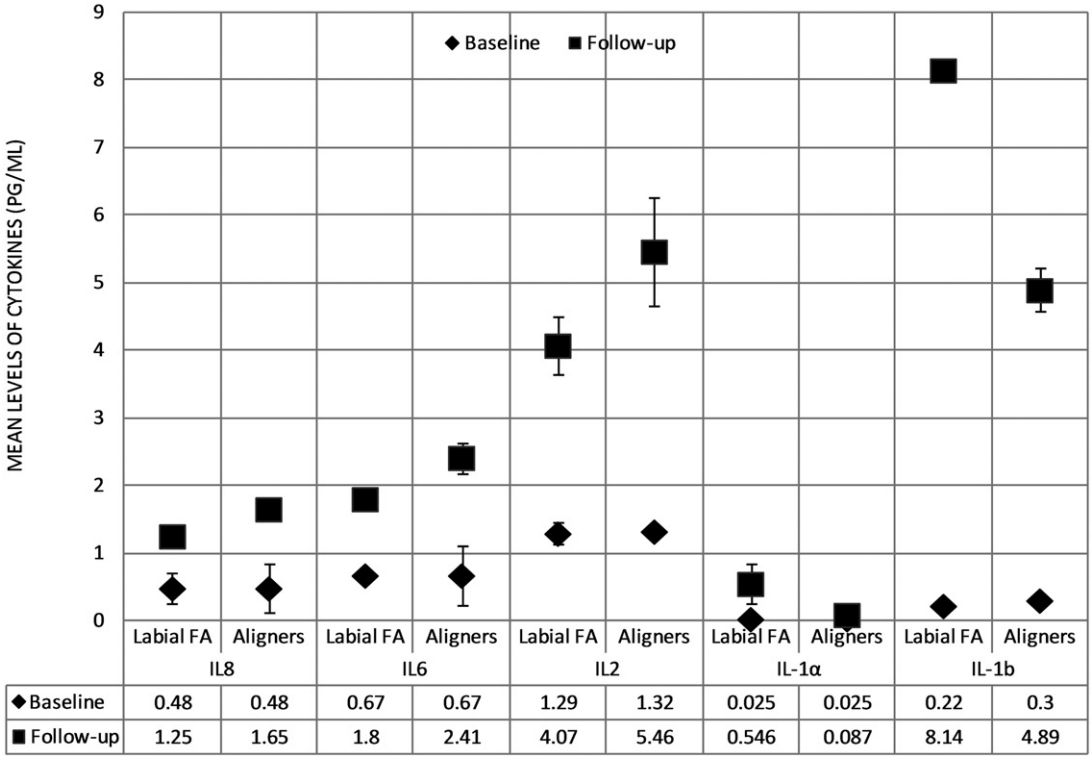
Figure 1. Mean and range levels of clinical parameters of plaque index (PI), gingival index (GI), bleeding on probing (BOP) within labial fixed appliances ($n = 20$) and aligner groups ($n = 20$) at baseline and follow-up. All mean values are non-significant.

Comparisons of the changes from baseline to 3 weeks between the two groups

There was a statistically significant increase in the levels of IL-1α, IL-1ß, IL-2, IL-6, IL-8 and TNF-α ($p < .001$) in the GCF of teeth subjected to orthodontic forces by labial fixed appliance and aligners at the follow-up when compared to that at the baseline (Figures 2 and 3).

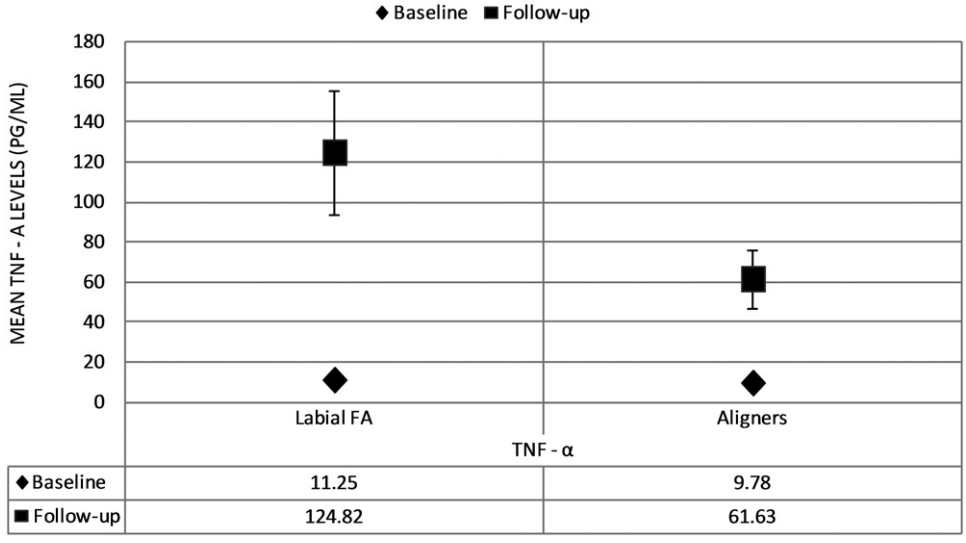
Most active cytokines

IL-1ß and TNF-α showed a prominent increase in their levels compared to the other cytokines in the GCF of teeth subjected to orthodontic forces by both the labial fixed appliance and aligners. The levels of IL-1ß and TNF-α were increased in patients treated with a labial fixed appliance compared to those treated by aligners, and the levels of IL-2, IL-6 and IL-8 were increased in patients treated by aligners



	IL8		IL6		IL2		IL-1α		IL-1b	
	Labial FA	Aligners	Labial FA	Aligners	Labial FA	Aligners	Labial FA	Aligners	Labial FA	Aligners
◆Baseline	0.48	0.48	0.67	0.67	1.29	1.32	0.025	0.025	0.22	0.3
■Follow-up	1.25	1.65	1.8	2.41	4.07	5.46	0.546	0.087	8.14	4.89

Figure 2. Mean and range levels of different cytokines with-in labial fixed appliances ($n = 20$) and aligner groups ($n = 20$) at baseline and follow-up. Range value of IL-1β for labial fixed appliances are: baseline = 0.255, follow-up = 32.80. All mean values are statistically significant.



	TNF - α	
	Labial FA	Aligners
◆Baseline	11.25	9.78
■Follow-up	124.82	61.63

Figure 3. Mean and range values of TNF-α values with labial fixed appliances ($n = 20$) and aligner groups ($n = 20$) at baseline and follow-up. All mean values are statistically significant.

compared to those treated by a labial fixed appliance (Figures 2 and 3).

Least active cytokine

IL-1α showed a minimal increase in its level compared to the other cytokines in the GCF of teeth subjected to orthodontic forces by both the labial fixed appliance and aligners (Figures 2 and 3). There was a statistically significant difference in the mean cytokine values of IL-1α, IL-2, IL-6, IL-8, TNF-α when compared between the labial fixed appliance and the aligners (Table 1). The mean cytokine values of IL-1β did not show any statistically significant difference when compared between the labial fixed appliance and aligner (Table 1).

Discussion

The orthodontic forces applied by the orthodontic appliances induce mechanical stimuli on the teeth, leading to an inflammatory response in periodontal tissues. Accordingly, inflammatory mediators get released in the GCF, which triggers biological processes associated with alveolar bone apposition and resorption [12–14]. Undoubtedly, the determination of the levels of various cytokines during orthodontic treatment helps us understand the underlying mechanism of bone metabolism [34]. The gingival fibroblasts produce inflammatory mediators such as chemokines, cytokines, proteolytic enzymes and prostaglandins actively take part in the inflammatory response and contribute to disease persistence [35–42].

The gingival sulcus was selected as the testing site because of its easy access to the oral cavity. The inflammatory response of the periodontal tissues due to the orthodontic tooth movement, gingivitis or the periodontitis leads to the release of cytokines in the GCF [6,33,43]. These cytokines act as biomarkers for various alveolar bone activities [44]. Osteoclast formation and bone resorbing activities are initiated by cytokines. IL-1, IL-2, IL-6, IL-8, TNF-α are the proinflammatory cytokines, and IL-4, IL-10 and IL-13 are anti-inflammatory cytokines [2,45]. IL-1ß is a major physiologic part of IL-1. IL-2 plays an active role in periodontal disease pathogenesis apart from stimulating osteoclastic activities [46,47]. It is considered a useful biomarker of inflammatory processes [19,32,48].

IL-6 stimulates osteoclast activities and causes bone resorption according to the literature available [24,49,50]. The level of IL-6 increased in the first 24 hours, and then, equilibrium was reached. Studies have shown that there was an increase in the levels of IL-6 only in the early stages, but there was no significant increase on days 7 and 21. This periodicity can possibly explain the fluctuation in the levels of IL-6 as seen in our study.

In a 28-day cycle, the levels of cytokines fluctuate [51–53]. IL-8 played a multifunctional role in the pathogenesis of periodontal diseases [52]. In periodontitis, the chemokines IL-8 attract neutrophils and other leukocytes to the inflammation site. IL-8 is secreted by various cells, including monocytes, lymphocytes, epithelial cells, endothelial cells, and fibroblasts, in response to IL-1, TNF-α [35]. In a study done by Basaran et al. [24], the orthodontic forces evoked proinflammatory cytokines changes, which led to bone resorption, and there was an increase in the levels of IL-2, IL-6 and IL-8.

Despite the fact that orthodontic treatment with Invisalign aligner is a widely used treatment option, apart from non-extraction treatment of mild to moderate malocclusions of non-growing patients, no clear recommendations about other indications of the system can be made, based on solid scientific evidence. Invisalign might treat faster mild non-extraction cases, but it requires more time than fixed appliance treatment for more complex cases. Invisalign aligners can safely straighten dental arches in terms of levelling and derotating the teeth. The orthodontic forces produced by the aligners are intermittent in nature. According to Kuncio et al. [53], the typical stages of tooth movement as described by Krishnan and Davidovitch were not shown by the teeth moved by aligners due to the intermittent forces provided by it. These forces produce less cell damage in the periodontium [54–56].

The inflammatory cytokines IL-1 and TNFα play a prominent role in the pathogenesis of periodontitis [25]. At the cellular level, these two cytokines are involved in the induction of several other inflammatory mediators, such as IL-6, IL-8, matrix metalloproteinases (MMPs) and PGE2 [34,57,58]. Basaran et al. [24] showed that the levels of IL-1ß and TNF-α were increased after levelling and distalization in the periodontal tissues. Other studies showed that IL-1α levels were increased in the early stage of treatment but were not significant [59,60]. TNFα is also involved at an early stage in the inflammatory cascade, as it is released from mast cells in response to bacterial challenge [35]. A study performed by Castroflorio et al. [1] showed that there was an increase in the level of IL-1ß on the pressure side. The study by Sharath et al. [61] showed that IL-1ß was more expressed due to the mechanical stresses from orthodontic forces applied to the periodontium. Our results were similar to these findings, in which the levels of IL-1ß and TNF-α were prominently increased compared to the other cytokines in the GCF. IL-1β levels reached a peak concentration on the 21st day after orthodontic activation with Invisalign in the study conducted by Chami et al. [62], Iwasaki et al. [63] and Castrofolio et al. [1], which was similar to the results of our study.

A study conducted by Miethke and Vogt [14], on a comparison of the periodontal health of patients during treatment with the Invisalign system and with fixed orthodontic appliances, stated that the plaque index was significantly higher in patients treated with fixed labial appliances as compared to aligners.

Levrini et al. [64] conducted a study on the periodontal health status in patients treated with the Invisalign system and fixed orthodontic appliances; a 3 months clinical and microbiological evaluation. The results of their study concluded that the periodontal health status was better in patients treated with removable aligners compared to patients treated with fixed appliances. The removable appliances facilitated oral hygiene procedures. The Invisalign

group showed the absence of periodontal pathogenic bacteria, whereas the fixed appliance group showed a higher number of periodontal pathogenic bacteria.

The study conducted by Abbate et al. [10], on the periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances, showed that the patients treated with removable appliances demonstrated better compliance with oral hygiene and presented less plaque and gingival inflammatory reactions as compared to those treated with fixed appliances.

The release of the cytokines we have studied is due to the mechanotransduction occurring during the orthodontic tooth movement [65–71]. Although many studies have been done on the cytokine productions during orthodontic treatment but there were differences in the appliance design and the forces been used [36,72]. In the present study, there was an increase in the levels of cytokines in different proportions. Perhaps it occurred due to our methods which caused mechanical stimuli immediately after the placement of orthodontic appliances [73,74]. Many individuals vary in the biologic responses to mechanical loading. Bone mineral density, age, sex, anatomic structure or cellular activity in alveolar bone and periodontal ligament could be the related differences [75–77].

The bias that GCF is sensitive to saliva contamination and plaque should be considered. The bone, blood vessels, gingiva, etc. could act as sources for cytokines. Considering the wide range of interfering factors and biologic response in both GCF and periodontal ligament, the number of subjects participating in this study may be a limiting factor. The changes in cytokine levels after orthodontic treatment with both the labial fixed appliances and aligners over a longer period of treatment time could not be studied, whereas a shorter duration of 3 weeks was considered which is also the limitation of our study. Further studies should therefore be carried out with a larger sample size and on a longer duration of treatment.

Conclusions

The levels of the six studied cytokines in GCF (IL-1α, IL-1ß, IL-2, IL-6, IL-8 and TNF-α) increased after 3 weeks both after treatment with the conventional labial fixed appliance and with aligners. Some of the cytokines increased more, some less. However, there were only minor differences in the changes of the cytokine levels from baseline to 3 weeks between the two groups.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

[1] Castroflorio T, Gamerro EF, Caviglia GP. Biochemical markers of bone metabolism during early orthodontic tooth movement with aligners. Angle Orthod. 2017;87:74–81.

[2] Rody WJ, Wijegunasinghe M, Wiltshire WA, et al. Differences in the gingival crevicular fluid composition between adults and adolescents undergoing orthodontic treatment. Angle Orthod. 2014;84:120–126.

[3] Alfano MC. The origin of gingival fluid. J Theor Biol. 1974;47:127–136.

[4] Lamster IB. The host response in gingival crevicular fluid: potential applications in periodontitis clinical trials. J Periodontol. 1992;63:1117–1123.

[5] McCulloch CA. Host enzymes in gingival crevicular fluid as diagnostic indicators of periodontitis. J Clin Periodontol. 1994;21:497–506.

[6] Angel F, Remya K, Sajitha K. Levels of interleukin -10 in gingival crevicular fluid and its role in the initiation and progression of gingivitis to periodontitis. Oral Hyg Health. 2014;2:1–7.

[7] Surlin P, Rauten AM, Silosi I, et al. Pentraxin-3 levels in gingival crevicular fluid during orthodontic tooth movement in young and adult patients. Angle Orthod. 2012;82:833–838.

[8] Grieve WG, Johnson GK, Moore RN, et al. Prostaglandin E (PGE) and interleukin-1 beta (IL-1 beta) levels in gingival crevicular fluid during human orthodontic tooth movement. Am J Orthod Dentofacial Orthop. 1994;105:369–374.

[9] Lombardo L, Arreghini A, Ramina F, et al. Predictability of orthodontic movement with orthodontic aligners: a retrospective study. Prog Orthod. 2017;18:35–47.

[10] Abbate GM, Caria MP, Montanari P, et al. Periodontal health in teenagers treated with removable aligners and fixed orthodontic appliances. J Orofac Orthop. 2015;76:240–250.

[11] Karkhanechi M, Chow D, Sipkin J, et al. Periodontal status of adult patients treated with fixed buccal appliances and removable aligners over one year of active orthodontic therapy. Angle Orthodontist. 2013;83:146–151.

[12] Rossini G, Parrini S, Castroflorio T, et al. Periodontal health during clear aligners treatment: a systematic review. Eur J Orthod. 2015;37:539–543.

[13] Levrini L, Novara F, Margherini S, et al. Scanning electron microscopy analysis of the growth of dental plaque on the surfaces of removable orthodontic aligners after the use of different cleaning methods. Clin Cosmetic Investig Dent. 2015;7:125–131.

[14] Miethke RR, Vogt S. A comparison of the periodontal health of patients during treatment with the Invisalign system and with fixed orthodontic appliances. J Orofac Orthop. 2005;66:219–229.

[15] Zheng M, Liu R, Ni Z, et al. Efficiency, effectiveness and treatment stability of clear aligners: a systematic review and meta-analysis. Orthod Craniofac Res. 2017;20:127–133.

[16] Harrison J. Orthodontic treatment. Vital. 2011;8:31–35.

[17] Benson PE, Javidi H, DiBiase AT. What is the value of orthodontic treatment? Br Dent J. 2015;218:185–190.

[18] Statistics ND. NHS Dental Statistics for England: 2011/12. In: Issue 2012. Health and Social Care Information Centre, Prescribing & Primary Care.

[19] Davies TM, Shaw WC, Worthington HV, et al. The effect of orthodontic treatment on plaque and gingivitis. Am J Orthod Dentofacial Orthop. 1991;99:155–161.

[20] Mohlin B, Axelsson S, Paulin G, et al. TMD in relation to malocclusion and orthodontic treatment. Angle Orthod. 2007;77:542–548.

[21] Gelgör IE, Karaman A, Ercan E. Prevalence of malocclusion among adolescents in central anatolia. Eur J Dent. 2007;1:125–131.

[22] Sidlauskas A, Lopatiene K. The prevalence of malocclusion among 7-15 year old Lithuanian school children. Medicina (Kaunas). 2009;45:147–152.

[23] Perinetti G, Paolantonio M, D'Attilio M, et al. Alkaline phosphatase activity in gingival crevicular fluid during human orthodontic tooth movement. Am J Orthod. 2002;122:548–556.

[24] Başaran G, Ozer T, Kaya FA, et al. Interleukine-1beta and tumor necrosis factor-alpha levels in the human gingival sulcus during orthodontic treatment. Angle Orthod. 2006;76:830–836.

[25] Alfaqeeh SA, Anil S. Gingival crevicular fluid flow rate and alkaline phosphatase level as potential marker of active tooth movement. Oral Health Dent Manag. 2015;13:458–463.

[26] Almeida RC, Capelli JJ, Teles RP. Levels of gingival crevicular fluid matrix metalloproteinases in periodontally compromised teeth under orthodontic forces. Angle Orthod. 2015;85:1009–1014.
[27] Canavarro C, Teles RP, Capelli JJ. The monitoring of gingival crevicular fluid volume during orthodontic treatment: a longitudinal randomized split-mouth study. Eur J Orthod. 2013;34:109–113.
[28] Canavarro C, Teles RP, Capelli JJ. Matrix metalloproteinases -1, -2, -3, -7, -8, -12, and -13 in gingival crevicular fluid during orthodontic tooth movement: a longitudinal randomized split-mouth study. Eur J Orthod. 2013;35:652–658.
[29] Nassrawin NA. Detection of ostecalcin in gingival crevicular fluid in a group of orthodontic patients. J Int Soc Prev Community Dent. 2018;8:168–173.
[30] Jamesha FI, Maradi IP, Chithresan K, et al. Comparison of gingival crevicular fluid periostin levels in healthy, chronic periodonotitis, and aggressive periodontitis. J Indian Soc Periodontol. 2018;22: 480–486.
[31] Yadav N, Lamba AK, Thakur A, et al. Effect of periodontal therapy on lactoferrin levels in gingival crevicular fluid. Aust Dent J. 2014; 59:314–320.
[32] Davidovitch Z. Tooth movement. Crit Rev Oral Biol Med. 1991;2: 411–450.
[33] Sueda T, Bang J, Cimasoni G. Collection of gingival fluid for quantitative analysis. J Dent Res. 1969;48:159.
[34] Chowdhary A, Gayathri GV, Mehta DS. Comparative analysis of GCF beta-glucuronidase level in diabetic and nondiabetic patients with chronic periodontitis: a clinicobiochemical study. J Indian Soc Periodontol. 2008;12:16–20.
[35] Stoycheva MS, Murdjeva MA. Correlation between serum levels of interleukin-1β, interleukin- 1RA, interleukin-6, interleukin-10, interleukin 12, tumor necrosis factor-α and interferon-γ with some clinical and laboratory parameters in patients with salmonellosis. Biotechnol Equip. 2005;19:143–146.
[36] Basaran G, Ozer T, Kaya FA, et al. Interleukins 2, 6, and 8 levels in human gingival sulcus during orthodontic treatment. Am J Orthod Dentofacial Orthop. 2006;130:7.e1–7.e6.
[37] Yucel-Lindberg T, Båge T. Inflammatory mediators in the pathogenesis of periodontitis. Expert Rev Mol Med. 2013;15:e7.
[38] Flavell SJ, Hou TZ, Lax S, et al. Fibroblasts as novel therapeutic targets in chronic inflammation. Br J Pharmacol. 2009;153: S241–S246.
[39] Heath JK, Atkinson SJ, Hembry RM, et al. Bacterial antigens induce collagenase and prostaglandin E2 synthesis in human gingival fibroblasts through a primary effect on circulating mononuclear cells. Infect Immun. 1987;55:2148–2154.
[40] Meikle MC, Hembry RM, Holley J, et al. Immunolocalization of matrix metalloproteinases and TIMP-1 (tissue inhibitor of metalloproteinases) in human gingival tissues from periodontitis patients. J Periodontal Res. 1994;29:118–126.
[41] Reynolds JJ, Meikle MC. Mechanisms of connective tissue matrix destruction in periodontitis. Periodontol 2000. 1997;14:144–157.
[42] Båge T, Lindberg J, Lundeberg J, et al. Signal pathways JNK and NF kappa B, identified by global gene expression profiling, are involved in regulation of TNFalpha-induced mPGES-1 and COX-2 expression in gingival fibroblasts. BMC Genomics. 2010;11: 241–250.
[43] Davidovitch Z, Finkelson MD, Steigman S, et al. Electric currents, bone remodeling, and orthodontic tooth movement. I. The effect of electric currents on periodontal cyclic nucleotides. Am J Orthod. 1980;77:14–32.
[44] Davidovitch Z, Finkelson MD, Steigman S, et al. Electric currents, bone remodeling, and orthodontic tooth movement. II. Increase in rate of tooth movement and periodontal cyclic nucleotide levels by combined force and electric current. Am J Orthod. 1980; 77:33–47.
[45] Al-Ghurabi BH, Mohammed-Salih HS, Ghazi A, et al. Evaluation of salivary levels of proinflammatory cytokines (IL1α, IL-8 and GM-CSF) in adult orthodontic patients. Iosrjdms. 2014;13:75–78.
[46] Bankers-Fulbright JL, Kalli KR, McKean DJ. Interleukin-1 signal transduction. Life Sci. 1996;59:61–83.
[47] Yamaguchi M. RANK/RANKL/OPG during orthodontic tooth movement. Orthod Craniofac Res. 2009;12:113–119.
[48] Blich AM, Tamisc NO, Walid A, et al. The effect of using different types of orthodontic forces upon the level of interleukin II in the human gingival crevicular fluid (clinical study). J Dent Health Oral Disord Ther. 2015;2:00070.
[49] Scarel-Caminaga RM, Trevilatto PC, Souza AP, et al. Investigation of an IL-2 polymorphism in patients with different levels of chronic periodontitis. J Clin Periodontol. 2002;29:587–591.
[50] Papadimitriou A, Mousoulea S, Ganditis N, et al. Clinical effectiveness of Invisalign orthodontic treatment: a systematic review. Prog Orthod. 2018;19:37–45.
[51] John S, Turner D, Donn R, et al. Two novel biallelic polymorphisms in the IL-2 gene. Eur J Immunogenet. 1998;25:419–420.
[52] Alhashimi N, Frithiof L, Brudvik P, et al. Orthodontic tooth movement and de novo synthesis of proinflammatory cytokines. Am J Orthod Dentofacial Orthop. 2001;119:307–312.
[53] Kuncio D, Maganzini A, Shelton C, et al. Invisalign and traditional orthodontic treatment postretention outcomes compared using the American board of orthodontics objective grading system. Angle Orthod. 2007;77:864–869.
[54] Davidovitch Z, Nicolay OF, Ngan PW, et al. Neurotransmitters, cytokines, and the control of alveolar bone remodeling in orthodontics. Dent Clin North Am. 1988;32:411–435.
[55] Lee KJ, Park YC, Yu HS, et al. Effects of continuous and interrupted orthodontic force on interleukin-1beta and prostaglandin E2 production in gingival crevicular fluid. Am J Orthod Dentofacial Orthop. 2004;125:168–177.
[56] Nakao K, Goto T, Gunjigake KK, et al. Intermittent force induces high RANKL expression in human periodontal ligament cells. J Dent Res. 2007;86:623–628.
[57] Tuncer BB, Ozmeric N, Tuncer C, et al. Levels of interleukin-8 during tooth movement. Angle Orthod. 2005;75:631–636.
[58] Samuels RH, Pender N, Last KS. The effects of orthodontic tooth movement on the glycosaminoglycan components of gingival crevicular fluid. J Clin Periodontol. 1993;20:371–377.
[59] Baik HS, Kim CK, Lim WH, et al. Interleukin-1α and tumor necrosis factor-α expression on the compressed side of gingiva during orthodontic tooth movement. Ojst. 2012;02:182–187.
[60] de Aguiar MC, Perinetti G, Capelli J. The gingival crevicular fluid as a source of biomarkers to enhance efficiency of orthodontic and functional treatment of growing patients. Biomed Res Int. 2017;2017:1.
[61] Shetty SK, Kumar M, Smitha PL. Cytokines and orthodontic tooth movement. J Dent Sci Res. 2011;2:132–141.
[62] Chami VO, Nunes L, Capelli JJ. Expression of cytokines in gingival crevicular fluid associated with tooth movement induced by aligners: a pilot study. Dental Press J Orthod. 201;23:41–46.
[63] Iwasaki LR, Chandler JR, Marx DB, et al. IL-1 gene polymorphisms, secretion in gingival crevicular fluid, and speed of human orthodontic tooth movement. Orthod Craniofac Res. 2009;12:129–140.
[64] Levrini L, Mangano A, Montanari P, et al. Periodontal health status in patients treated with the Invisalign® system and fixed orthodontic appliances: a 3 months clinical and microbiological evaluation. Eur J Dent. 2015;9:404–410.
[65] Ren Y, Maltha JC, Kuijpers-Jagtman AM. Optimum force magnitude for orthodontic tooth movement: a systematic literature review. Angle Orthod. 2003;73:86–92.
[66] Okada N, Kobayashi M, Mugikura K, et al. Interleukin-6 production in human fibroblasts derived from periodontal tissues is differentially regulated by cytokines and a glucocorticoid. J Periodontal Res. 1997;32:559–569.
[67] Krishnan V, Davidovitch Z. Cellular, molecular, and tissue-level reactions to orthodontic force. Am J Orthod Dentofacial Orthop. 2006;129:469e1–46932.
[68] Krishnan V, Davidovitch Z. On a path to unfolding the biological mechanisms of orthodontic tooth movement. J Dent Res. 2009; 88:597–608.
[69] Yamaguchi M, Ozawa Y, Mishima H, et al. Substance P increases production of proinflammatory cytokines and formation of

osteoclasts in dental pulp fibroblasts in patients with severe orthodontic root resorption. Am J Orthod Dentofacial Orthop. 2008;133:690–698.

[70] Andrade I, Silvana R, Taddei SR, et al. Inflammation and tooth movement: the role of cytokines, chemokines, and growth factors. Semin Orthod. 2012;18:257–269.

[71] Ren Y, Vissink A. Cytokines in crevicular fluid and orthodontic tooth movement. Eur J Oral Sci. 2008;116:89–97.

[72] Silness J, Løe H. Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condtion. Acta Odontol Scand. 1964;22:121–135.

[73] Alhashimi N, Frithiof L, Brudvik P, et al. Orthodontic movement induces high numbers of cells expressing IFN-gamma at mRNA and protein levels. J Interferon Cytokine Res. 2000;20:7–12.

[74] Kohara H, Kitaura H, Yoshimatsu M, et al. Inhibitory effect of interferon-gamma on experimental tooth movement in mice. J Interferon Cytokine Res. 2012;32:426–431.

[75] Reitan K. Tissue behavior during orthodontic tooth movement. Am J Orthod. 1960;46:881–900.

[76] Reitan K. Clinical and histologic observations on tooth movement during and after orthodontic treatment. Am J Orthod. 1967;53:721–745.

[77] Shiau HJ, Aichelmann-Reidy ME, Reynolds MA. Influence of sex steroids on inflammation and bone metabolism. Periodontol 2000. 2014;64:81–94.