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ORIGINAL ARTICLE

Detection of gingival crevicular fluid cytokines in children and adolescents with and without fixed orthodontic appliances

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Abstract

Objective. To study the expression of IL-1β, IL-4, and IL-8 in the gingival crevicular fluid (GCF) of children, adolescents, and young adults with and without fixed orthodontic appliances. ***Material and methods***. Eighty systemically healthy children and adolescents participated in the study: 56 aged between 8 and 16 years without any orthodontic appliance (Group A) and 24 aged between 10 and 20 years having worn fixed orthodontic appliances for at least 12 months (Group B). Clinical examination included presence or absence of plaque, probing depth, bleeding on probing, and gingival overgrowth. GCF was collected by means of Durapore strips from four randomly selected sites per subject. The contents of interleukin-1 beta (IL-1β), interleukin-4 (IL-4), and interleukin-8 (IL-8) were detected by ELISA, measured as total amounts (pg/30s) and expressed in log scale. ***Results***. Statistically significant differences were noted for the mean log IL-1β, IL-4, and IL-8 between the two groups: Group B showed significantly higher mean levels in log IL-1β and log IL-8 compared to Group A. Mean levels of log IL-4 were lower in Group B, although they did not reach statistical significance. Furthermore, mean levels of log IL-1β and log IL-8 were associated with bleeding sites ($p < 0.001$) and gingival overgrowth, while mean level of log IL-4 was associated with non-bleeding sites and no gingival overgrowth ($p < 0.001$). ***Conclusion***. Our findings suggest that fixed orthodontic appliances result in an increase in the expression of IL-1β and IL-8. This may reflect biologic activity in the periodontium during orthodontic tooth movement.

Key Words: *Cytokines, gingival crevicular fluid, orthodontic treatment*

Introduction

Gingival health is compromised during orthodontic treatment. A local change is produced in the oral ecosystem altering the composition of bacterial plaque and resulting in the development of an inflammatory process [1–3]. Inflammation in periodontal tissue is almost inevitable when fixed orthodontic appliances are used [4,5]. These may render oral hygiene difficult even for the most motivated patients [6]. In the past, the effects of fixed orthodontic appliances on marginal periodontal tissue were analyzed predominantly by means of clinical and microbiological studies [7]. Clinically, an increase in plaque scores, gingival scores, and probing depths has been reported [1,8]. These clinical observations are attributed to the alteration of the composition of the bacterial biofilm occurring soon after the placement of fixed orthodontic appliances. In most cases, there is a shift in the subgingival microflora to a periopathogenic population with increases in anaerobic rods and reductions in facultative anaerobes [9–11].

Another way of studying the changes occurring during orthodontic tooth movement is by analysis of the composition of the gingival crevicular fluid (GCF). GCF reflects the immune and inflammatory reactions deriving from host-parasite interactions [12,13] and bio-mechanical stress [14–17]. It is a non-invasive method, and until now a wide variety of substances involved in bone remodeling, and produced by the PDL cells in sufficient amounts to diffuse into the GCF, has already been studied [18]. Among many inflammatory and immune mediators identified in GCF, cytokines have attracted particular attention. For example, the level of interleukin-1 beta (IL-1β), a known potent cytokine produced

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mainly by activated monocytes increases significantly during inflammation [19] and participates in the initiation of bone resorption [20,21]. Interleukin-4 (IL-4), originally described as B-cell growth factor, is a potent downregulator of macrophage function. Localized absence of IL-4 in diseased periodontal tissues was associated with periodontal disease activity and progression [22]. Finally, Interleukin-8 (IL-8) is produced by a wide variety of cells (polymorphonuclear leukocytes, monocytes, macrophages, and fibroblasts) and plays a key role in the accumulation of leukocytes at the sites of inflammation [23].

The aim of the present study was to evaluate the clinical and biochemical characteristics accompanying the inflammatory process of periodontal tissues around fixed orthodontic appliances in children, adolescents, and young adults. The levels of IL-1β, IL-4, and IL-8 in GCF of children undergoing orthodontic treatment are compared to those without orthodontic appliances.

Material and methods

Sample size

An allocation ratio of 1:2 was used for better representation of the wide range of age groups. A sample size of 23 and 46 subjects for each group, respectively, achieves 90% power to detect a pattern of IL-1β and IL-4 means, equal to 2.2 and 2.5 log × pg/30s with a standard deviation of 0.35 log × pg/30s and level of significance equal to a = 0.05. Similarly, for IL-8, a pattern of means equal to 3.2 and 3.5 log × pg/30s will be detected with the same number of subjects for each group, respectively, and under the same assumptions.

Subjects

A total of 80 healthy children, adolescents, and young adults participated in the study. They were selected from two private dental clinics in Piraeus, Greece limited to pedodontics and periodontics, respectively. All were systemically healthy and had not received antibiotics in the 6 months prior to entering the study. Before the beginning of the study, all subjects underwent a session of supragingival scaling and received oral hygiene instructions. On the day of the examination they presented no non-treated carious lesions. The subjects were divided into two groups: Group A of 56 children and adolescents (32 M, 24 F, mean age 12.3 ± 2.9) with healthy periodontium showing no radiographic evidence of bone loss and a probing pocket depth <2 mm. Group B of 24 adolescents and young adults (15 M, 9 F, mean age 15.1 ± 2.9) wearing fixed orthodontic appliances for at least 12 months and showing no radiographic evidence of bone loss.

The qualified subjects were entered consecutively in the study. All participants (parents of children and adolescents) gave their consent to participation. The study was conducted in accordance with the principles outlined in the Helsinki declaration of 1975 (as revised in 1983) on experimentation involving human subjects.

Periodontal examination

The clinical evaluation was performed by one periodontist (J.K.) and included the presence or absence of plaque (PI) [24], assessment of probing pocket depth (PPD), and bleeding upon probing (BOP) [25] at four sites around each tooth, excluding 3rd molars. The presence or absence of inflammatory gingival overgrowth (GO) was also assessed. Measurements of PPD were carried out to the nearest millimeter using a Goldman/Fox Williams periodontal probe. The number of teeth present was recorded for each child.

Investigator calibration

A total of 10 periodontally healthy adolescents and young adults who were not included in the study group were used for the calibration evaluation. The single designated examiner (J.K.) performed full mouth PPD and CAL measurements for all 10 subjects and repeated the measurements after 15 min. The intra-examiner STD for repeated measures was 0.1 mm. Examiner's reproducibility was 99.8%.

Gingival crevicular fluid sampling

All teeth within a quadrant were numbered from 1 to 7. One tooth and the respective mesial or distal site from each quadrant were randomly sampled using the random number generator of SPSS 13.0. The GCF was collected from four sites in each child–adolescent–young adult by means of Durapore filter membranes (pore size = 0.22 mm; Millipore Corp., Bedford, Mass., USA). After isolation of the test sites from saliva, a first Durapore strip was inserted 1 mm into the sulcus and left in place for 15 s. Three minutes after removal of the first strip, a second Durapore strip was similarly inserted in the same site for 15 s. The two strips were then placed in a microcentrifuge tube and immediately frozen at −20°C until the day of the analysis. In the event of visible contamination with blood, the strips were discarded.

Clinical measurements were recorded and GCF sampling sites were pre-selected one week before sampling. Clinical parameters were registered again after GCF sampling and these values were used in the analysis.

Analysis of cytokine production

The samples were transferred to and analyzed blindly by C.G. in the Department of Periodontology at the University of Geneva. The contents of IL-1β, IL-4, and IL-8, were measured at each of the four sites from each patient. A total of 320 samples in 80 subjects were analyzed. On the day of the analysis, 350 μl of phosphate-buffered saline (PBS, pH 7.2) was added to the tubes containing the strips. The strips were gently shaken for 1 min and then centrifuged at 2000*g* for 5 min, with the strips kept at the collar of the tube in order to elute GCF components completely. After strip removal, the supernatant was divided into three aliquots for determination of each biochemical compound. The amounts of IL-1β, IL-4, and IL-8 were determined by enzyme-linked immunoadsorbent assays (ELISA) specific for each compound (Ruwag Diagnostics, Zürich, Switzerland). The assays were carried out in accordance with the manufacturer's instructions and the levels of the biochemical compounds were reported as total amount (pg) per 30 s sample and were expressed in log scale.

Total cytokine amounts per 30 s samples were calculated based on ELISA concentration values. Sites with cytokine levels below the limits of the assay's detectability were scored as 0 pg.

Data analysis

Statistical analyses were carried out on the full set of patients who participated in the study. Baseline demographic and clinical characteristics were summarized by the mean and standard deviation (±SD) for continuous measures and the number and percent for categorical variables. Differences of demographic data and probing depth between groups were tested using a *t*-test. Differences between genders and differences in plaque, bleeding on probing, and gingival overgrowth levels between groups were tested using the chi-squared test. A mixed model analysis for the logarithms of IL-1β, IL-4, and IL-8 was made using a random intercept for describing the correlation between observations from the same subject. Differences in log IL-1β, log IL-4, and log IL-8 means between groups, genders, bleeding on probing, and gingival overgrowth were tested using appropriate F-tests based on REML. However, the F-test degrees of freedom are not presented since they are taken into account from the corresponding *p*-values.

Results

Demographic and periodontal variables

The demographic and periodontal variables in each group are summarized in Table I. As expected, the mean PPD, the percentage of sites with bleeding on probing, and the presence of gingival overgrowth were significantly higher in subjects wearing orthodontic appliances (Group B) than in those without (Group A). The percentage of sites with plaque accumulation did not differ between the two groups.

Table I. Demographic and periodontal variables.

	Group A	Group B
Age	8–16	10–20
Mean (SD) age, years	12.3 (2.9)	15.1 (2.9)
Men/women	32/24	13/11
Mean probing depth (SD) at sampling sites	0.03 (0.19)	0.38 (0.49)**
Plaque[1]	71	68.8
Bleeding on probing[1] (BOP)	17	64.6#
Gingival overgrowth[1] (GO)	9.4	76#

[1]Percent positive sites. [2]Chi-squared, among groups <0.001.

Means of log IL-1β, IL-4, and IL-8 in Groups A and B

The mean (SD) total amounts of IL-1β, IL-4, and IL-8 in the two groups are given in Table II and the fitted log transformed means (SE) in Table III. Statistically significant differences were found for the mean log of IL-1β and IL-8 between the two groups ($F = 33.262$, $p < 0.001$ and $F = 4.054$, $p = 0.048$, respectively). The mean log of IL-4 was not statistically different between the groups ($F = 3.074$, $p = 0.083$).

No differences were noted between males and females for log IL-1β ($F = 1.979$, $p = 0.163$), for log IL-4 ($F = 3.158$, $p = 0.079$), or for log IL-8 ($F = 3.194$, $p = 0.078$) (data not shown in the tables).

Correlations with bleeding sites

Table IV gives possible associations between the mean log of IL-1β, log IL-4, and log IL-8 scores and BOP levels. Log IL-1β and log IL-8 were associated with the bleeding sites ($p < 0.001$), whereas IL-4 was associated with non-bleeding sites ($p < 0.001$).

Correlations with gingival overgrowth

Table V gives possible associations between the mean log IL-1β, log IL-4, and log IL-8 scores and presence or absence of gingival overgrowth expressed as 1 and 0, respectively. IL-1β and IL-8 were associated with gingival overgrowth ($p = 0.018$ and $p < 0.001$, respectively), whereas IL-4 was

Table II. Mean (SD) IL-1β, IL-4, and IL-8 total amounts (pg/30s) in patients without (Group A) and with (Group B) fixed orthodontic appliances.

	Group A	Group B
IL-1β	10.7 (4.44)	16.3 (3.88)
IL-4	12.2 (3.92)	10.5 (3.23)
IL-8	26.7 (5.56)	30.3 (8.35)

Table III. Fitted mean (SE) log of IL-1β, IL-4, and IL-8 in patients without (Group A) and with (Group B) fixed orthodontic appliances.

	Group A	Group B	F	*p*
IL-1β	2.29 (0.045)	2.76 (0.069)	33.262	<0.001
IL-4	2.44 (0.041)	2.31 (0.063)	3.074	0.083
IL-8	3.26 (0.030)	3.37 (0.045)	4.054	0.048

associated with sites without gingival overgrowth ($p < 0.001$).

Discussion

In the present study, the levels of IL-1β, IL-4, and IL-8 were monitored in the GCF of orthodontically treated young subjects and untreated controls. Total amounts rather than concentrations were used, because very small errors in volume determination can lead to large errors in estimates of final concentrations if the total volume collected is small. The results demonstrate that young adults with fixed orthodontic appliances had significantly higher levels of IL-1β and IL-8 compared to children and adolescents with healthy periodontium. The levels of IL-4 did not differ between the two groups. These differences between the groups were associated with the presence of bleeding on probing and the presence of inflammatory gingival overgrowth.

Oral hygiene was monitored before the day of examination. Ideal oral hygiene was important because the study aimed to investigate the differences on the levels of IL-1β, IL-4, and IL-8 in GCF, as a consequence of inflammation caused by tooth movement rather than plaque accumulation. In fact, plaque accumulation did not differ between the groups.

Several studies have reported that, during orthodontic treatment with a fixed appliance, an increase in the plaque accumulation and gingival inflammation is seen frequently. This can be attributed to the increased difficulty of effective cleaning around the appliance [5,6]. Regardless of the level of oral hygiene, when a fixed appliance is placed the majority of patients develop generalized gingivitis [26,27]. Histologically, the interdental gingival of banded teeth presents the pattern of an established gingival lesion: pronounced leukocyte infiltration and inflammatory exudation in the area of the transeptal fibers [28].

Table IV. Differences (δ) between BOP levels in the mean log IL-1β, log IL-4, log IL-8.

	IL-1β	IL-4	IL-8
δ BOP (1–0)	0.272	−0.227	0.260
F	35.092	20.937	64.983
p	<0.001	<0.001	<0.001

BOP = bleeding on probing. 0 = BOP negative. 1 = BOP positive.

Table V. Differences (δ) between GO levels in the mean log IL-1β, log IL-4, log IL-8.

	IL-1β	IL-4	IL-8
δ GO (1–0)	0.093	−0.159	0.119
t	5.638	13.066	15.119
p	0.018	<0.001	<0.001

GO = gingival overgrowth. 0 = GO negative. 1 = GO positive.

Furthermore, gingival enlargement occurs soon after placement of a fixed orthodontic appliance and explains the increase of probing depth found during treatment [8]. In our study, a significant increase in PPD was seen in the orthodontically treated patients. This increase could more properly be related to moderate gingival enlargement rather than attachment loss. It is well established that orthodontic appliances can influence the subgingival microbial population, i.e. "shifting" it to a more pathogenic population. This may in part explain the inflammation during orthodontic treatment even in patients with excellent plaque control [29].

In terms of the GCF metabolites, several studies have shown that during orthodontic treatment the application of mechanical force induces an inflammatory reaction by the compression of periodontal ligament [30–32]. As a result, both the GCF flow and its components are modified. Until now, a wide variety of substances have already been studied [18]. In our study, the differences on the GCF cytokine levels observed between the two groups reflect the events taking place in the periodontium during orthodontic tooth movement. Furthermore, these differences may reflect the age-associated changes in immunity, since there is a communication between the endocrine and immune system that may determine the expression of cytokines in puberty [33]. However, only a few studies have considered age status as a modifying factor for the variations in intracellular cytokine production. This variation was observed when comparing younger and aging adults or children and adolescents [33,34].

It has been proposed that the severity of gingivitis in childhood is linked to several factors, including puberty [35]. However, so far there is no evidence of a direct link between gingivitis and puberty. This is because the chronological age is a poor indicator of puberty, the measurement of gingivitis is subjective, and the hormone levels are not measured.

In conclusion, within the limitations of the study, the results indicate that fixed orthodontic appliances, as well as puberty, result in an increase in the expression of IL-1β and IL-8 which might reflect the host response to the orthodontically induced local inflammation.

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