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Differences in IL-1β and IL-6 levels in the gingival crevicular fluid during acute phase of orthodontic tooth movement between juveniles and young adults

Različiti nivoi citokina IL-1β i IL-6 u gingivalnoj tečnosti tokom početne faze ortodontskog pomeranja zuba kod dece i mladih

Amila Vujačić*, Aleksandra Konić†, Jasna Pavlović*, Vera Todorović‡, Vladanka Vukićević*, Danimir Jevremović‡, Nadežda Milošević-Jovčić†

***Department of Dentistry, Faculty of Medicine, University of Priština/Kosovska Mitrovica, Kosovska Mitrovica, Serbia; †Institute for Medical Research, University of Belgrade, Belgrade, Serbia; ‡Faculty of Stomatology, University of Busines Academy of Novi Sad, Pančevo, Serbia**

Abstract

Background/Aim. There is little information, about the difference in cytokine levels in the gingival crevicular fluid (GCF) during orthodontic tooth movement (OTM), between juveniles (children) and young adults (adults). The aim of this study was to examine the levels of interleukins IL-1β and IL-6 in GCF of these two age groups during the acute phase of OTM. **Methods.** The subjects, 10 children and 10 adults, underwent OTM of a single tooth, with an untreated antagonistic tooth used as the control group. GCF was sampled from both the control and treatment sites right before the beginning (the baseline) and 24 h, 72 h and 168 h upon initiation of OTM. Cytokine levels were determined by enzyme-linked immunosorbent assay (ELISA). **Results.** The levels of both GCF IL-1β and IL-6 showed a bimodal peak during early phase of OTM, at 24 h and 168 h, in both age groups. As the statistic has shown, the increase in IL-1β levels was more prominent after 168 h in treated teeth of children, compared to both children's control teeth and treated teeth of adults, whilst the GCF IL-6 levels in the same group increased significantly after 24 h, as well as after 168 h, approximately 70 and 55 fold, respectively. In the same time periods the increase of IL-6 levels in GCF of adults was notably lesser, averaging approximately 5 and 10 fold, respectively, compared to the control teeth. In addition, the amount of tooth movement was statistically larger for children than for adults 168 hours upon the initiation of OTM. **Conclusion.** GCF IL-1β and IL-6 were increasingly expressed during initial phase of OTM in both children and adults. However, excretory response of cytokines in children's GCF, especially the concentration of IL-6, was at a significantly higher level than that of adults', which accords to the finding that the initial OTM is faster in children.

Key words:
tooth movement; child; adolescent; adult; interleukin-6; interleukin-1 beta; gingival cervicular fluid; acute-phase proteins.

Apstrakt

Uvod/Cilj. Postoji malo podataka o razlikama u nivou citokina u gingivalnoj tečnosti (GT) kod dece i odraslih u toku ortodontskog pomeranja zuba (OPZ). Cilj ove studije bio je da se ispitaju koncentracije citokina IL-1β i IL-6 u GT kod dece i odraslih u toku rane faze OPZ. **Metode.** Ispitavanje je obuhvatilo dve grupe ispitanika – 10 dece i 10 odraslih osoba, kod kojih je postavljen ortodontski separator između drugog premolara i prvog molara na jednoj strani, a suprotna strana je služila kao kontrolna. Uzorci GT uzimani su i sa lečenih i sa kontrolnih zuba i to pre, 24 h, 72 h i 168 h nakon postavljanja separatora. Nivo citokina određivan je *enzyme-linked immunosorbent assay* (ELISA) metodom. **Rezultati.** Praćenje koncentracije IL-1β i IL-6 u GT u toku rane faze OPZ pokazalo je dva pika vrednosti za oba citokina u obe grupe ispitanika – 24 h i 168 h od postavljanja separatora. Međutim, porast vrednosti IL-1β bio je statistički značajno veći kod dece nakon 168 h u GT lečenih zuba u odnosu na kontrolne, kao i u GT lečenih zuba dece u odnosu na lečene zube odraslih ispitanika. Što se tiče koncentracije IL-6 u GT, ona je kod dece bila statistički značajno viša u GT lečenih zuba o odnosu na kontrolne vrednosti kako nakon 24 h posmatranja (povećanje od oko 70 puta), tako i nakon 168 h posmatranja (povećanje od oko 55 puta). Kod odraslih se zapažao isti trend u povećanju koncentracije ovog citokina u GT lečenih zuba u odnosu na kontrolne u posmatranim periodima, ali je to povećanje, iako statistički značajno, bilo manje (oko 5 puta posle 24 h i 10 puta posle 168 h posmatranja) u odnosu na

Correspondence to: Amila Vujačić, University of Priština, Faculty of Medicine, Department of Dentistry, Anri Dinana bb, 38 220 Kosovska Mitrovica, Serbia. E-mail: amilavujacic@gmail.com

isto kod dece. Uz to, stepen pomeranja zuba izražen u mm kod dece bio je statistički viši od istog kod odraslih ispitanika nakon 168 h od primenjenog ortodontskog lečenja. **Zaključak.** IL-1β i IL-6 se povećano luče u GT u toku rane faze OPZ kako kod dece, tako i kod odraslih ispitanika. Međutim, sekretorni odgovor, naročito u pogledu sekrecije IL-6, daleko je veći kod dece nego kod odraslih ispitanike, što je u skladu sa nalazom da je stepen početnog pomeranja zuba brži u ovoj populaciji ispitanika.

Ključne reči:
zub, pomeranje; deca; adolescenti; odrasle osobe; interleukin-6; interleukin-1 beta; gingivalna sulkusna tečnost; proteini akutne faze.

Introduction

During orthodontic correction of tooth position, the remodelling of periodontal ligament and alveolar bone takes place, in response to mechanical load. The early phase of this process is characterized by an aseptic inflammation. Various pro-inflammatory cytokines have been suggested to play their role in it. Inflammatory mediators may trigger the biological processes associated with alveolar bone resorption and apposition [1]. In order to study these factors in humans, non-invasive methods have been developed, which rely on results from gingival crevicular fluid (GCF) samples. The GCF content is presumed to reflect the physiological status of the periodontal ligament [2]. Interleukins (IL) IL-1β and IL-6 are some of the first cytokines to increase in levels in GCF, during the application of orthodontic force. IL-1β is a key mediator, involved in a variety of immune and acute-phase inflammatory response activities, having been detected in GCF during orthodontic tooth movement (OTM) [2–4]. IL-6 interacts directly with bone cells, playing an important role in the local regulation of bone remodelling, as well as in the acute inflammation associated with the OTM [5, 6]. IL-1β is an inducer of IL-6 [7], and they both participate in the complex mechanism of mediators that regulate inflammation.

Although numerous studies on concentration of various cytokines in GCF during OTM have been conducted, there is little data available on the effect of age on cytokine production in humans. The purpose of this work was to examine and compare the expression of IL-1β and IL-6 cytokines in GCF in children and adults, during the early phase of OTM.

Methods

Subjects

The subjects undergoing orthodontic treatment were 10 children (ages 9–14, mean age 13) and 10 adults (ages 19–24, mean age 20), without any health issues, selected according to following criteria: 1) good general health; 2) no antibiotic therapy within 3 months prior to the study; 3) no anti-inflammatory drugs, nor analgesics in the month preceding the study; 4) healthy periodontium with generalized probing depth of 2 mm. The study was performed with the informed consent of the adult patients and the children's parents, and was approved by the Ethics Committee of the Faculty of Medicine in Kosovska Mitrovica.

Application of force

Orthodontic elastic separator (Dentalastics separators blue 2.1 mm, Dentaurum, Germany) was inserted between the second premolar and the first permanent molar in the mandible (experimental site). The untreated antagonistic tooth served as the control group. Both control and experimental sites showed good periodontal status. The amount of tooth movement was measured by digital nonius, using new splint for each patient. The precision of nonius measurement was 0.1 mm.

GCF collection

GCF was sampled at the control and treatment sites right before (the baseline), 24 h, 72 h, and 168 h (the checkpoints) after initiation of orthodontic treatment. Paper strips were inserted into the gingival crevice for 60 s, then transferred into the plastic tubes and stored at -70 °C until use. GCF was eluted from each strip into 250 μL phosphate buffered saline (PBS) and extracted by 5 min centrifugation at 15 G.

Cytokine levels determination

Cytokines levels in GCF were determined using enzyme-linked immunosorbent assay (ELISA) kits specific for each cytokine (Quantikine® HS ELISA Assay, R&D systems Inc. USA), and reported as the total mass (in pg) *per* 60 s GCF sample. The lower detection limits were 0.125 pg/mL and 0.156 pg/mL for IL-1α and IL-6, respectively.

Statistics

Statistical analysis was performed using Mann-Whitney tests and SPSS for Windows $p < 0.05$ was considered significant.

Results

In general, analysis of GCF samples showed detectable amounts of both IL-1β and IL-6, in control and treated teeth of all subjects, throughout the observation period. Moreover, the presence of the IL-1β significantly exceeded that of IL-6 cytokines in all the examined fluid samples.

Control GCF concentrations of IL-1β and IL-6 in children and adults

Concentrations of IL-1β and IL-6 in GCF of the control teeth of children and of adults were similar (Table 1). Both IL-1β and IL-6 were present in measurable amounts in all GCF samples of the control teeth, in both examined groups. Generally, GCF IL-1β concentrations significantly exceeded those of IL-6 in both examined groups, in normal conditions. Mean values of GCF IL-1 β concentrations were between 8.5 pg/min and 10.3 pg/min, and 5.3 pg/min and 6.1 pg/min in the children and adult groups respectively, in observation period, while the mean values of GCF IL-6 concentrations were

Table 1

Control GCF concentrations of IL-1β and IL-6 in children and adults

Interleukin		Baseline	24 h	72 h	168 h	*p*
IL-1β (pg/60 sec)*	Children	8.9 ± 2.4	8.7 ± 2.4	10.3 ± 3.7	8.5 ± 2.4	n.s.
	Adult	6.0 ± 1.9	6.1 ± 1.1	6.0 ± 1.2	5.3 ± 2.2	
IL-6 (pg/60 sec)*	Children	0.03 ± 0.01	0.03 ± 0.00	0.04 ± 0.01	0.03 ± 0.00	n.s.
	Adult	0.05 ± 0.02	0.10 ± 0.03	0.10 ± 0.04	0.02 ± 0.00	

***Values were done as mean ± standard error; ns – non significant; GCF – gingival crevicular fluid; IL – interleukin.**

between 0.03 pg/min and 0.04 pg/min, and 0.02 pg/min and 0.1 pg/min in the children and adult groups, respectively, in the same period. The control values of IL-1 β in GCF were notably higher in children than in adults in all time intervals, but these differences were not statistically significant. However, control GCF IL-6 values were somewhat lower in children than in adults in the same time intervals, also without statistical significance.

GCF concentrations of IL-1β in children and adults during the acute phase of OTM

The concentrations of IL-1 β in GCF of children and adults are shown in Figure 1. Our results indicate a bimodal peak of IL-1β levels in GCF during the early phase of OTM, in both children and adult groups: the baseline concentrations increased 24 h and 168 h after the application of the separator. In addition, by comparison, this increase was more prominent in adults group after 24 h, but in children's group after 168 h. However, 24 h into OTM, the peak IL-1β value of the children group exceeded the said value in the adults group, but this difference was not statistically significant. The only statistically significant difference in GCF IL-1β concentrations was at 168 h between the control and treated teeth of the children ($p < 0.05$).

The comparison of GCF concentrations of IL-1β of orthodontically treated teeth of children and adults is offered in Figure 2. The higher concentrations of IL-1β in GCF of treated teeth were first observed in children, then in adults, at

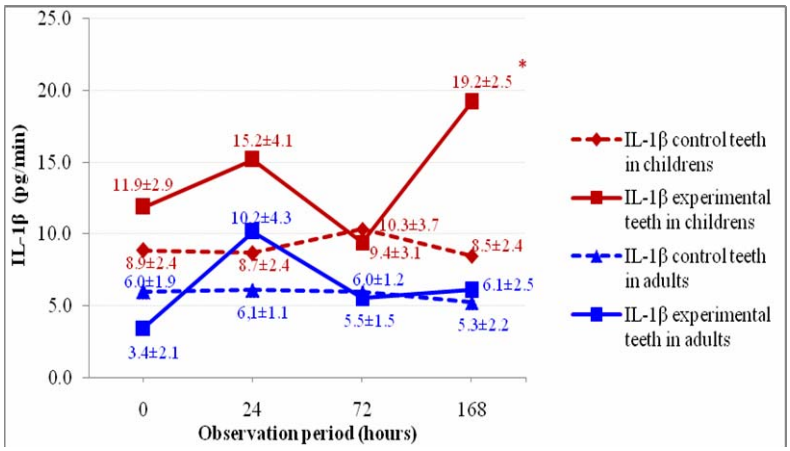

Fig. 1 – Gingival crevicular fluid (GCF) concentrations of interleukin-1β (IL-1β) in children and adults during the acute phase of orthodontic tooth muvement (OTM); *$p < 0.05$ [treated (experimental) teeth in children *vs* control teeth in children].

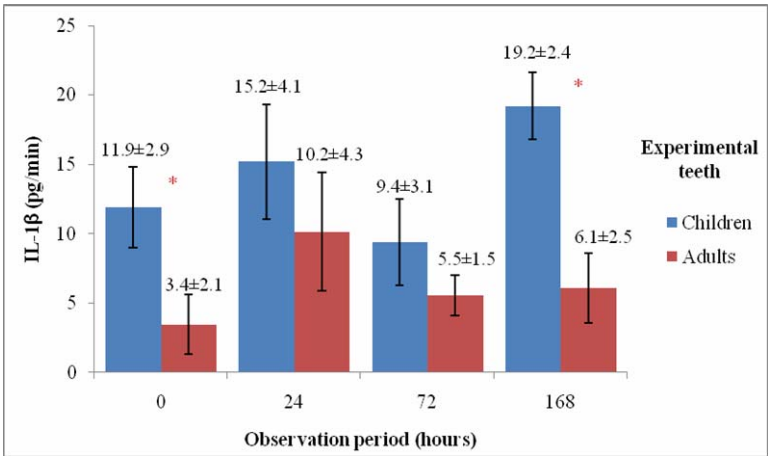

Fig. 2 – Gingival crevicular fluid (GCF) concentrations of interleukin-1β (IL-1β) during the acute phase of orthodontic tooth movements (OTM); *$p < 0.05$ (children *vs* adults).

all checkpoints, but the only statistically significant difference was at the baseline and again at 168 h. Apparently, children had a statistically significantly higher level of IL-1β at the starting point of the experiment ($p < 0.05$), as well as 168 h into OTM ($p < 0.05$).

GCF concentrations of IL-6 in children and adults during acute phase of OTM

The concentrations of IL-6 in GCF in children and adults are shown in Figure 3. Mechanical load applied to tooth triggered a significant release of IL-6 24 h upon initiation ($p < 0.05$), as well as after 168 h ($p < 0.05$), in both examined groups. Peak values at 24 h were higher than at 168 h. Moreover, in addition to statistically significant differences of IL-6 concentrations in GCF between the treated and the control teeth in both examined age groups, in the said checkpoints, higher concentrations were found in the children group. Whilst the GCF levels of IL-6 in the children group increased at 24 h and 168 h approximately by 70 and 55 fold, respectively, in adults they increased approximately by 5 and 10 fold, respectively compared to the control teeth (data not shown). Also, statistically significantly higher IL-6 concentrations in GCF of treated teeth were observed 72 h into OTM only in the children group ($p < 0.05$), approximately a 10 fold increase, compared to the corresponding control teeth (data not shown).

When we compared IL-6 concentration in GCF of treated teeth in adults to those in children, the higher concentrations of IL-6 were observed in children at all checkpoints, but the only statistically significant differences were at 72 h and 168 h ($p < 0.05$) (Figure 4).

The amount of tooth movement after OTM

The amount of tooth movement for children (1.08 ± 0.04 mm) was larger than for adults (0.89 ± 0.04) after 168 h of acute phase of OTM. This difference was statistically significant ($p < 0.003$) (Figure 5).

Correlation between the GCF levels of IL-1b and IL-6 and the velocity of tooth movement after acute phase of OTM

A positive nonsignificant correlation between the levels of both cytokines in GCF and the average velocity of tooth movement was evident in children, while in adults such correlation was registered only for IL-1β (Figure 6).

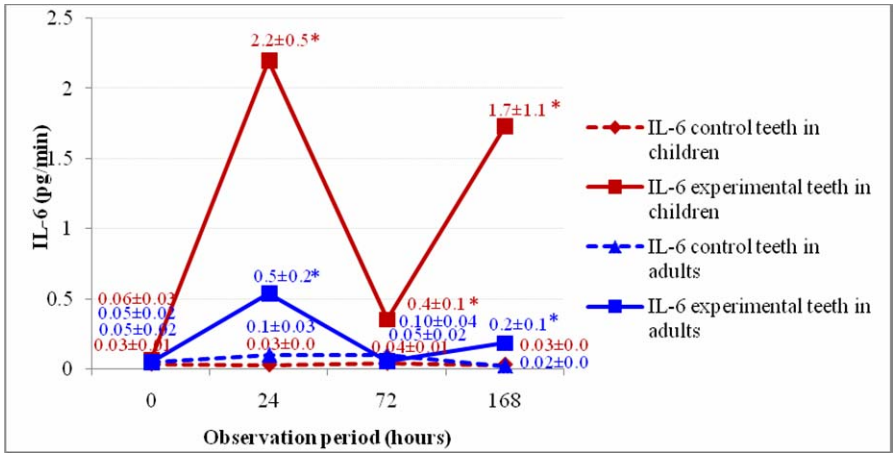

Fig. 3 –Gingival crevicular fluid (GCF) concentracions of interleukin-6 (IL-6) in children and adults during the acute phase of orthodontic tooth movements (OTM); *$p < 0.05$ (children *vs* adults).

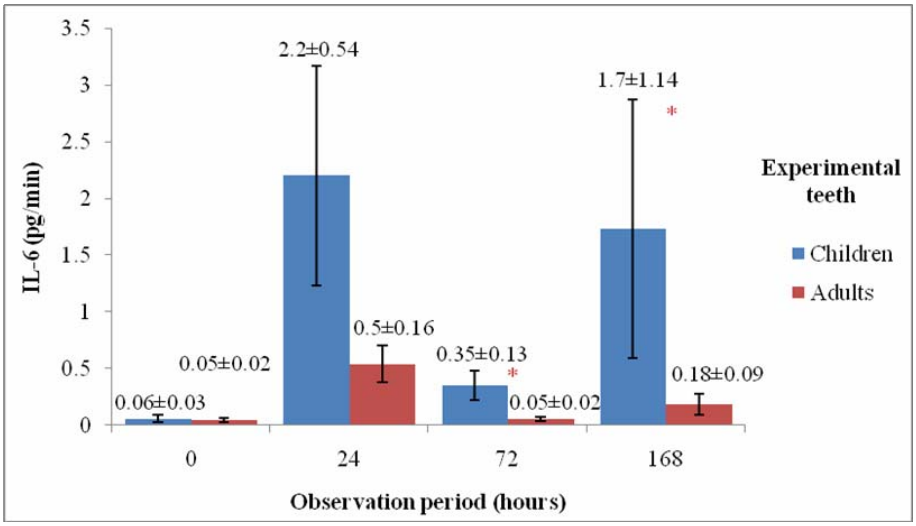

Fig. 4 – Gingival crevicular fluid (GCF) concentrations of interleukin-6 (IL-6β) during the acute phase of orthodontic tooth movements (OTM); *$p < 0.05$ (children *vs* adults).

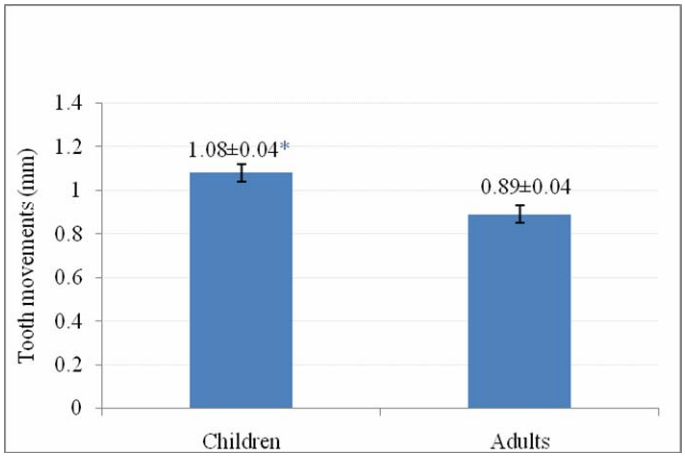

Fig. 5 – Tooth movements (mm) at 168 h after acute phase of orthodontic tooth movements (OTM) in children and adults,*$p < 0.003$ (children *vs* adults).

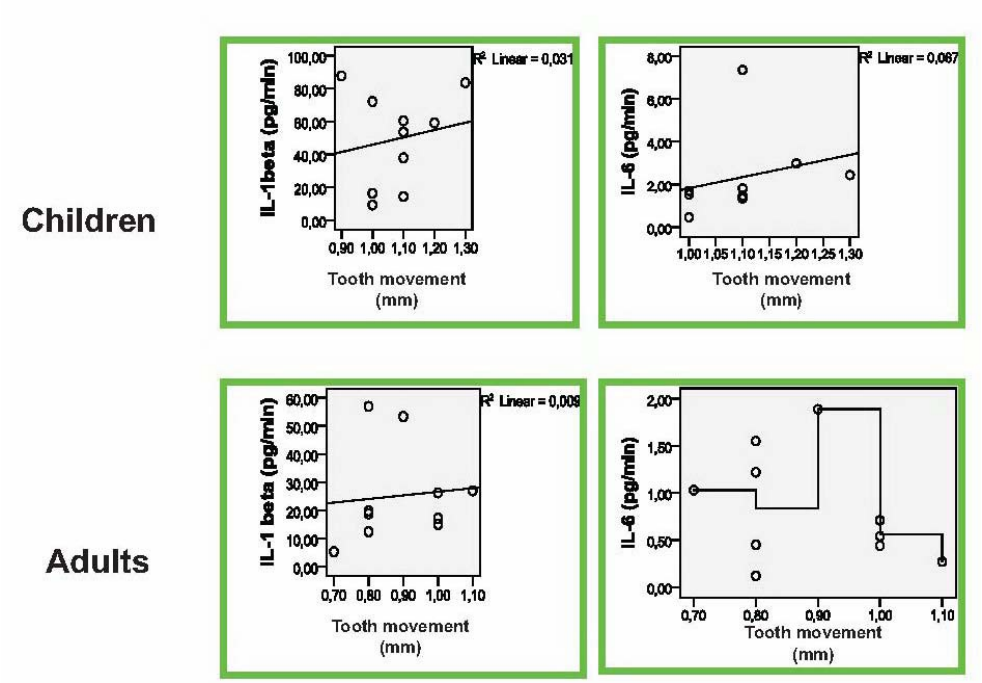

Fig. 6 – Correlation between cytokine levels and the velocity of tooth movement.

Discussion

Age has to be considered as a contributing factor compromising the remodelling potential, *ie* inflammatory response of parodontium during OTM, but the information about cytokine levels and other signal molecules in the GCF regarding patients of different age are sparse. Some of the previous studies demonstrated variations in the levels of different cytokines and signal molecules, including IL-1β, IL-4, IL-6, IL-8, GM-CSF, prostaglandin E2 (PGE2), receptor activator of NF κB ligand (RANKL), osteoprotegerin (OPG) and pentraxin-3, regarding age and orthodontic activation period correlation. Those studies might explain why the speed of orthodontics treatment differs between children and adults [8–13] to such a large extent. The present study was designed to evaluate changes in expression of IL-1β and IL-6 in GCF during the early phase of orthodontic treatment, the difference in this process between children and adults (different age groups), as well as its effects on amount of tooth movement in these two groups.

The contents of IL-1β and IL-6, as detected by ELISA, were measured as total cytokine mass *per* GCF volume secreted in 60 s *per* strip, and expressed in pg/60 s. We believe, as do some other authors, that in the described manner the amount of cytokines in GCF, secreted by periodontal tissues, is presented most realistically [11, 14], and taken most accurately, considering the specific need for the concentration to be expressed in pg/μL GCF or pg/g protein of GCF [15].

The contents of the IL-1β significantly exceeded those of IL-6 cytokines in the fluid of both control and treated teeth, both in children and adults, as had also been reported in other studies [2, 16–18]. Our results indicate that IL-1 β levels in GCFs during the early phase of OTM showed a bimodal

peak in both children and adult groups, but after 24 h this increase was more prominent in the adults group, but after 168 h it was in children. There were statistically significant higher levels of IL-1β in the very beginning of OTM, as well as 168 h from the beginning of treatment, in GCF samples of children's treated teeth, in comparison to those of adults. Our findings are consistent with previously reported data on increased levels of proinflammatory cytokines, including IL-1β and IL-6, in GCF during human OTM [2, 8, 9, 16–18]. In the present study, we found that the content of IL-1β in GCF increased 24 h into OTM in both children and adults, this being the most consistent result reported in the literature [2, 3, 10–15]. However, individual variations of IL-1β in GCF levels were very high, for which reason the only statistically significant difference in concentration of IL-1β in GCF was the one occurring after 168 h between the treated and the control teeth in the children's group. Results of other studies indicate that equivalent force systems during OTM induce individually different cytokine production, which correlates with individual differences in the velocity of canine retraction [15]. There is evidence that IL-1β, IL-1 receptor antagonist (IL-1RA) and IL-1 gene polymorphisms probably play a part in OTM. The speed of tooth movement is related to stress and levels of IL-1β, IL-1RA and IL-1 gene polymorphisms in GCF [19–21]. Three factors that significantly affected the speed and provided the best predictive model for effective teeth movement were: activity index [AI = experimental (IL-1β/IL-1RA)/control (IL-1beta/IL-1RA)], concentration of IL-1RA in GCF and genotype of IL-1B [22].

The source of IL-1β, as well as other cytokines and regulatory molecules, in GCF, during OTM, may more likely come from the compressed periodontal ligament, the resorbing bone adjacent to the root surface or the adjacent gingiva. Hence patients involved in orthodontic treatment belong to different age groups, the age has to be taken into consideration as a contributing factor compromising the remodelling potential of periodontal tissues, as proposed in sparse publications. One recent study proposed that decreased periodontal ligament (PDL) metabolic activity is the reason for lower protein expression of signal molecule including fibroblast growth factor-basic (bFGF), fibroblast growth factor receptor 1 (FGFR1), IL-6, matix metalloproteinase 8 (MMP-8), and matix metaloproteinase 9 (MMP-9) in older patients, and that activity of remodelling process of periodontal tissue decreases with aging and expression of signalling molecule decreases in adults [23]. An explanation of data regarding the statistically significant rise of IL-1β level in children at the start and after 168 h of OTM, compared to the adult group, as well as the explanation to why the peak IL-1β value in 24 h of OTM of the group of children exceeds the equivalent value of adults', lies within the above mentioned finding that there is increased PDL metabolic activity in younger patients [17], and that the advanced level of IL-1β in GCF reflects higher cell activity in the periodontium during OTM [11]. It must be emphasized once again that in our study the second peak of IL-1β after 168 hours of OTM was registered only in the group of children.

IL-6 also showed the highest peak 24 h after placing the separator, in both groups, with a statistical significance, the increase in relation to the initial values being much higher in children than in adults. When we compared IL-6 concentration in GCF of the treated teeth between the adults and the children, the higher concentrations of IL-6 were observed in the later, throughout the observation period, but the only statistically significant differences were after 72 h and 168 h. The explanation for such results could be found in reports of other researchers, stating that inflammatory mediator levels, including IL-6, advance quicker in children than in adults [9].

Although varying in the quantitative level, the observed changes of the two cytokines are matched time-wise. This finding is mostly accordant to the data available in the literature, especially those considering the group (not all authors have examined both cytokines simultaneously), showing that the level of IL-1β and IL-6 both increases rapidly 24 hours after placing the separator [2, 3, 9, 14, 16, 17, 24–27]. Placing separators led to the early inflammatory response of local tissue, which is consistent with the generally accepted view of acute inflammation as a driving force of the process that leads to the remodelling of the periodontal tissues upon mechanical stress. Released at the site of inflammation, whether directly or indirectly (through the substance affected by the synthesis and secretion), they react with bone cells, initiating the process of bone resorption [6, 27–29].

It is known that IL-1β affects the initiation of IL-6 [5], so an increase in the content of IL-6, along the line of increasing IL-1β level, may be due to the described effect of IL-1β. IL-6 is generally considered a proinflammatory cytokine, and it is possible that the increase of its concentration 24 h after the initiation of orthodontic treatment was in function of mediation in the process of acute inflammation. Finding that IL-6 has increased many times over, shows that it is possible this cytokine is indeed part of the feedback mechanism regulating value of IL-1β.

It turned out that teeth movement in relation to the initial position was statistically higher in children (1.08 ± 0.11 mm) than in adults (0.89 ± 0.12 mm). Therefore, a positive correlation, though not statistically significant, was drawn between the tooth movement and the content levels of IL-6 and IL-1β, in children. In the group adults the trend was observed only for IL-1β. There is not much data on the effect of the concentration of cytokines on the degree and rate of tooth movement in the literature. Iwasaki et al. [15] had showed that there is a positive correlation of contents of IL-1β and the rate of tooth movement *per* day, having observed seven patients (mean age 13 years) for 84 days. The trend is somewhat consistent with our finding that in a 7-day period the content levels of IL-1β correlated with the amount of tooth movement. Under identical conditions, large interindividual differences occur regarding the speed of movement of the teeth [30], in both humans and animals, including genetically homogeneous individuals [15–17, 22, 31–38]. For differences in the degree of tooth movement between children and adults, where orthodontically treated teeth of children manifested significantly greater movement than those of adults, we have no clear explanation. Despite the occasional doubts about the effectiveness of orthodontic treatment in adults, clinical experience has shown that the movement of teeth through alveolar bone in adults is workable, but requires more time [38]. The opinion is that adults' biological ability to move the

teeth is reduced by one-third in comparison to that of children's [10, 39, 40]. This assertion is based on thoroughly familiar limitations of adult biological bones, the composition of which changes with aging, in reflection of the cells becoming less reactive, due to the slowing of metabolism.

Conclusion

GCF IL-1β and IL-6 were increasingly expressed during the initial phase of OTM both in children and adults. However, the levels of these cytokines, especially IL-6 concentrations, advance quicker in juveniles than in young adults, which concurs the finding that the initial OTM in juveniles is faster than in adults.

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