MATRIX METALLOPROTEINASE-8 AND INTERLEUKIN-1β IN GINGIVAL FLUID OF CHILDREN IN THE FIRST THREE MONTHS OF ORTHODONTIC TREATMENT WITH FIXED APPLIANCES

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ABSTRACT
In orthodontic treatment remodeling of periodontal space is concomitant with the movement of teeth. Matrix metalloproteinase-8 and interleukin-1β are key markers of the initial tissue reaction in the processes of remodeling. The gingival fluid is the medium where changes in the profile and levels of these mediators occur.

The aim of this study was to investigate the levels of matrix metalloproteinase-8 and interleukin-1β in gingival fluid samples during the first 3 months of orthodontic treatment with fixed appliances in children.

Materials and methods: Twelve children receiving brackets treatment were included in the study; 48 samples of gingival fluid collected from one representative tooth of these children were measured once before placing the brackets, then at 24 hours, at 1 week, and at 3 months. We measured the amount of gingival fluid and the levels of matrix metalloproteinase-8 and interleukin-1β.

Filter paper strips were used to collect gingival fluid. After eluting them we made a quantitative analysis of the biomarkers, matrix metalloproteinase-8 and interleukin-1β, using the solid-phase enzyme immunoassay (ELISA).

The results showed a slight drop in the levels of both markers compared with baseline values and an increase at three months of orthodontic treatment. A similar tendency was observed in the flow of gingival fluid.

Conclusion: Quantitative analysis of matrix metalloproteinase-8 and interleukin-1β in gingival fluid samples is potentially a non-invasive method by which orthodontists can get information about the remodeling processes in the periodontium during orthodontic treatment thus controlling and limiting them within physiological boundaries.

Key words: gingival crevicular fluid, matrix metalloproteinase-8, interleukin-1β, orthodontic tooth movement, periodontium remodeling

INTRODUCTION
The processes of periodontium remodeling are incident to orthodontic tooth movement.1-4 The gingival crevicular fluid (GCF) is the medium where changes in the profile and levels of various mediators of tissue reactions in tooth movement occur during orthodontic treatment.5-9

Prostaglandins, interleukins (IL)-1β, IL-6, TNF-α, epidermal growth factor (EGF), TNF-alpha and the receptor activator of nuclear factor kappa B ligand (RANKL), acid phosphatase, tartrate resistant acid phosphatase, cathepsin B, etc. can be found in GCF in elevated levels,10-16

Matrix metalloproteinases (MMPs) and their inhibitors (tissue inhibitors of matrix metalloproteinases, TIMPs) act in coordination to control the collagen remodeling in the periodontium. The expression of MMP-2, 8, 9, 13 and TIMPs 1-3 is upregulated during orthodontic tooth movement,3,4,7,17
One of the most efficient enzymes causing collagen decomposition (of type I collagen) in the course of inflammatory response and during tissue remodeling in orthodontic tooth movement is MMP-8 (collagenase 2). Physiologically, periodontal ligaments are constantly in a process of remodeling, but during orthodontic tooth movement there is a considerably more intense metabolism of the periodontal collagen.\textsuperscript{4,7,17-19}

IL-1β is another important marker of the initial tissue reaction in the processes of remodeling in the course of orthodontic treatment. Besides its inflammatory effect, it exerts a stimulating effect on bone resorption by activating the osteoclasts.\textsuperscript{6,9-11,14,16,20,21} Cytokines IL-6, TNF-a and EGF have the same function. IL-1β acts in synchrony with TNF-a, while the latter stimulates bone resorption and bone cell replication. IL-1 is also considered to be a potent inducer of IL-6 production.\textsuperscript{14} IL-1b is thought to be a key mediator in bone remodeling, and a number of recent trials have provided evidence in this regard.\textsuperscript{6,9-11,13,14,16,20,21}

The prevailing opinion is that analysing IL-1β and MMP-8 in samples of gingival fluid can constitute an appropriate non-invasive method to control and sustain the tissue changes within normal limits.\textsuperscript{6,7,10,11,13,14,16-23}

The advanced capabilities the modern molecular and genetic methods provide to study micro quantities of cellular mediators in GCF allow researchers to investigate the remodeling processes in an orthodontic treatment and control their effect in the course of the tooth movement.

The aim of this study was to measure MMP-8 and IL-1β levels in gingival fluid samples during the first 3 months of treatment with fixed orthodontic technique in children.

Tasks:
1. To record oral hygiene and gingival status during the first 3 months of orthodontic treatment in children;
2. To quantify GCF of representative teeth over the study period;
3. To determine the MMP-8 levels in GCF of representative teeth during the study;
4. To determine the IL-1β levels in GCF of representative teeth during the investigation period.

MATERIALS AND METHODS

PATIENTS

The study included 12 children (6 boys and 6 girls) aged 11 through 15. The inclusion criteria were as follows:

- Patients with upcoming orthodontic treatment with fixed technique consulted and treated in the Department of Orthodontics, Faculty of Dental Medicine, Sofia;
- good general health;
- no antibiotic therapy over the previous six months;
- no anti-inflammatory drugs received in the month preceding the study;
- periodontal health with indicators of probing to a depth of gingival pockets ≤ 3 mm and radiographic evidence of lack of periodontal bone loss.

The study was authorized by the Ethics Committee of Scientific Research with the Medical University in Sofia; informed consent was obtained by all patients personally or by their parents if minors.

The dental health status of the children was recorded using a special evaluation card which we developed on the basis of the child examination card adopted by the Department of Pediatric Dentistry, Faculty of Dentistry – Sofia. It included: risk assessment of caries, risk assessment of periodontal disease, dental and periodontal status. The following indices were used: oral hygiene index (OHI), based on the presence of dental plaque after staining using data from the entire dentition; Papilla Bleeding Index (PBI) of Saxer Mulhemen and determining palatal pocket depth by probing at four points (medial, vestibular, distal and oral). The orthodontic status was recorded based on the orthodontic analysis made by the orthodontist at the Department of Orthodontics of Faculty of Dental Medicine – MU Sofia. Prior to placing the brackets the following was documented: type of orthodontic deformity and abnormal position of teeth.

All clinical parameters were evaluated six times during the first 3 months of orthodontic treatment, of which this study will use four:
- First visit - before the beginning of orthodontic treatment (up to 1 week);
- Second visit – 24 hours after placing the brackets;
- Third visit - 1 week after visit 2;
- Fourth visit – at 3 months.

All tested parameters will be the subject of a
comprehensive prospective study during a two-year orthodontic treatment and will be subject of future publications.

Preventive control during orthodontic treatment
The group of children with orthodontic treatment with fixed appliance was subjected to a complex prophylactic program, based on risk assessment of caries, and including, beside the traditional scheme, the following:

- professional oral hygiene before initiation of treatment and at six weeks and three months;
- periodic monitoring at each visit and record of dental and periodontal status;
- motivation and training in proper oral hygiene with appropriate means for oral hygiene with brackets;
- remotivation and remonitoring of oral hygiene habits;
- work with parents.

Samples of gingival fluid
Tests were performed on 48 samples of gingival fluid, collected from a representative tooth prior to mounting the brackets, then at 24 hours, 1 week, and at 3 months. The following teeth were selected: first molar (in 4 children), central incisor (in 4 children), canine tooth (in 4 children). The amount of gingival fluid and the levels of MMP-8 and IL-1β were examined.

Examination of GCF
For the collection of GCF strips of rectangular filter paper, 2 mm by 12 mm, were used (FILPAP s.r.o., CZ-411 08 Steti, Czech Republic; medium fast). Before examination the dry test strips were weighted on an analytical scale together with polypropylene capsules of the Eppendorf type in which they were placed.

The test strips were placed for 3 minutes in the medial vestibular areas of the gingival sulcus until one feels slight resistance in the sulcus, with the teeth isolated from saliva and carefully dried. To avoid contamination of samples with blood and other contaminants, all clinical measurements were performed after collection of samples. After the tests, the strips were placed in mini Eppendorf capsules and were weighted again on an analytical scale thus measuring the amount of GCF. These samples were frozen at -30°C until the time of analysis.

Quantification of MMP-8 and IL-1β with ELISA
After thawing the samples and extracting the GCF (by our own technique) quantitative determination of examined markers was carried out in the clinical laboratory of Academic Ivan Penchev Dental University hospital by solid-phase ELISA. Highly sensitive kits for human MMP-8 and IL-1β in biological fluids were used (Human Matrix Metalloproteinase-8 ELISA, BioVendor; Human IL-1β Platinum ELISA BMS224/2 / BMS224/2TEN Bioscience). The results were recorded on Multiscan plus ELISA reader with 450 nm wavelength.

Statistical analysis
SPSS 17 was used for the statistical analysis; we obtained means and standard deviations (mean ± SD) of the studied parameters and compared them using the paired-sample t-test. The level of significance was set at p < 0.05.

Results
OHI and PBI of the children studied during the 4 visits
All indices we studied characterize the overall oral hygiene and gingival status of the study sample during the first three months of orthodontic treatment in their 4 visits (before, at 24 hours, at 1 week, at 3 months after placing the brackets). The results are shown in Table 1.

Table 1. Mean values of OHI and PBI at different visits of the surveyed children

<table>
<thead>
<tr>
<th>Indices</th>
<th>1st visit mean ± SD</th>
<th>2nd visit mean ± SD</th>
<th>3rd visit mean ± SD</th>
<th>4th visit mean ± SD</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>OHI</td>
<td>1.83 ± 0.37</td>
<td>1.19 ± 0.52</td>
<td>1.89 ± 0.58</td>
<td>1.73 ± 0.42</td>
<td>t1,2  = 3.81</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>t2,3  = 3.73</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>t3,4  = 1.14</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>t1,4  = 0.78</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>PBI</td>
<td>1.07 ± 0.60</td>
<td>0.56 ± 0.29</td>
<td>0.73 ± 0.33</td>
<td>0.50 ± 0.25</td>
<td>t1,2  = 5.17</td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>t2,3  = 2.46</td>
<td>p &lt; 0.05</td>
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<tr>
<td></td>
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<td></td>
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<td></td>
<td>t3,4  = 2.50</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>t1,4  = 3.47</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>
All children were found to have a poor oral hygiene during the first examination prior to orthodontic treatment. After professional oral hygiene was administered, OHI decreased significantly. OHI restored its mean values after one week and these were maintained until the third month despite the repeated professional oral hygiene (after the first month) and re-motivation of patients at each visit.

One week after professional oral hygiene and immediately after placing the brackets, the PBI decreased by half. There was a marked tendency towards maintaining significantly lower mean values of PBI, which are maintained until the third month. This indicates a slight gingival inflammation during the controlled period, regardless of poor oral hygiene.

**Amounts of GCF in the Examined Samples**
The amount of gingival fluid in the 48 samples taken from 12 patients during the 4 visits for the first 3 months of orthodontic treatment are presented in Table 2.

Mean baseline amount of GCF from a tooth is 1.079 ± 0.706 μl; one week after professional oral hygiene and 24 hours after mounting the brackets it decreases to 0.886 ± 0.672 μl and the amount is retained until the third month when it starts to rise. However, differences in mean values of GCF at the 4 measurements are not statistically significant (p > 0.05). A more extensive study of the dynamics of GCF during orthodontic tooth movement is to come.

### Table 2. GCF amounts at different visits (μl)

<table>
<thead>
<tr>
<th>GCF</th>
<th>Visit 1</th>
<th>Visit 2</th>
<th>Visit 3</th>
<th>Visit 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean ± SD</td>
<td>1.079 ± 0.706</td>
<td>0.886 ± 0.672</td>
<td>0.883 ± 0.501</td>
<td>1.03 ± 0.464</td>
</tr>
<tr>
<td>t</td>
<td>t1,2 = 0.933</td>
<td>t2,3 = 0.024</td>
<td>t3,4 = -1.827</td>
<td>t1,4 = -0.361</td>
</tr>
<tr>
<td>p</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
<td>p &gt; 0.05</td>
</tr>
</tbody>
</table>

**Figure 1.** Amounts of IL-1β in samples of GCF (pg/ml).

**Levels of IL-1β in GCF during the first 3 months of orthodontic treatment**
The results of the levels of IL-1β in GCF samples are shown in Fig. 1.

The mean baseline values of IL-1β at baseline were 112.89 ± 94.67 pg/ml, which agrees with the results from other studies, as most authors have reported levels based on total protein in the fraction (19.2 pg6; 0.88 ± 0.11 pg/μg14; 0.58 ± 0.08 pg/μg16).

During the first two visits after placing the brackets and during the first week, they do not change significantly, but at 3 months the values increase significantly (p < 0.05).

**Levels of MMP-8 in GCF during the first 3 months of orthodontic treatment**
The results obtained from a quantitative study of MMP-8 in samples of gingival fluid in the 4 studies are shown in Fig. 2.

Mean values of MMP-8 before mounting the brackets and carrying out professional oral hygiene were 8.11 ± 3.12 ng/ml, while immediately after placing them there was a slight downward shift, but it failed to reach statistical significance (p > 0.05). At three months, however, a significant increase in the mean MMP-8 levels in GCF was found reaching 8.73 ± 2.06 ng/ml (p < 0.05). The MMP-8 levels in GCF were also within the limits as reported by different authors (36 ± 18.32 μg/l4; 56 ± 50 4.6 ± 4 μg/l19; 173 ± 145 ng/ml23,24), which with no clinical evidence of gingival inflam-
mation may be associated with the process of bone remodeling during orthodontic tooth movement.

**DISCUSSION**

The effect of orthodontic treatment on the amount of GCF has been studied for a long time with the results still contradictory. Some studies have found increased amounts during treatment. According to other studies, the increased amount of GCF that is caused by orthodontically moved teeth occurs earlier than the changes in GCF composition.

Our results show slow changes in the GCF amount during the first 3 months of treatment, but still there is an increase in the amount of GCF at three months. This tendency has yet to be studied on a larger number of samples.

In the present study a methodology for collecting samples of GCF and measuring micro amounts of IL-1β and MMP-8 in them by solid phase ELISA is adapted for the first time in this country. The results indicate that gingival fluid can be used as an excellent non-invasive diagnostic medium for testing basic tissue remodeling markers (MMP-8 and IL-1β) as reported in literature.

The values we get for the biomarkers we study are within the range of those reported in similar studies published in recent years.

However, the values of the studied biomarkers from different studies are hard to compare because of the different methodologies used in them - the immunofluorescent method that is considered more sensitive than ELISA, different types of ELISA and other methods.

It is noteworthy that both markers decrease slightly compared with the baseline values and increase at three months of orthodontic treatment. Similar studies report results indicating a rise in the amount of MMP-8 in the initial stages of treatment. These are studies with a few subjects for a very short period of follow-up (8 hours, 1 week, up to a month after the beginning of orthodontic treatment, involving single teeth of 5, 8, 11 patients). In other tests carried out in animal experiments, the above was not reported to such an extent and only slight increase in the levels of MMP-8 was found.

Some authors have found increased levels of IL-1β in samples of GCF on the very first day after placing the brackets and these were sustained until 48 hours, and even until 168 hours. These are results of short-term studies with a small sample (a week of research in 6, 9, 10, 12 children). A large part of the reported results are obtained after application of specific force in the specific area under study (e.g. a canine in retraction phase). Moreover, it was found that there are no such changes in other teeth included in the arc, and application of light forces does not lead to any significant increases in IL-1β.

Tracking of MMP-8 and IL-1β markers for a longer period of treatment, in a larger group of patients, and for various groups of teeth is limited in available literature. This gives us grounds for future research which we intend to carry out over the entire orthodontic treatment.

Our results for the slight increase of both the GCF flow and the markers tested at 3 months, but not at the beginning, may be the evidence for a well-planned treatment with application of gradually rising forces of impact which do not cause rapid stress in periodontal structures in comparison with the next step of the alignment phase (placing a stronger arc), application of greater forces of action which cause an increase of the levels of the studied markers in GCF.

**CONCLUSIONS**

1. Regardless of professional oral hygiene control in the first three months of orthodontic treatment, poor oral hygiene combined with minimal gingival inflammation is observed.
2. The levels of both markers (MMP-8 and IL-1β) decrease slightly compared with baseline values and show an increase to the third month of orthodontic treatment.
3. A similar tendency is observed in the amount of gingival crevicular fluid.
4. The studied biomarkers are within the limits of those reported in similar studies recently.

The quantitative analysis of MMP-8 and IL-1β in GCF samples can potentially be a non-invasive method by which information can be obtained about the processes of remodeling of the periodontium during orthodontic treatment and using which these processes can be controlled and limited in physiological limits.

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REFERENCES


МАТРИКС МЕТАЛЛОПРОТЕИНАЗА-8 И ИНТЕРЛЕЙКИН–1β В ГИНГИВАЛЬНОЙ ЖИДКОСТИ У ДЕТЕЙ В ТЕЧЕНИЕ ПЕРВЫХ ТРЕХ МЕСЯЦЕВ ОРТОДОНТИЧЕСКОГО ЛЕЧЕНИЯ. ИЗУЧЕНИЕ С ПОМОЩЬЮ ФИКСИРОВАННОЙ ТЕХНИКИ
Л. Рибагин, М. Рашкова

РЕЗЮМЕ
ВВЕДЕНИЕ: При ортодонтическом лечении процессы ремоделирования периодONTALного пространства сопутствуют движение зубов. Матрикс металло-протеиназа-8 и интерлейкин-1β представляют основные маркеры начальной реакции ткани при процессах ремоделирования. Гингивальная жидкость - это среда, в которой наблюдаются изменения в профиле и уровнях этих медиаторов.

Цель: Настоящее исследование ставит себе цель изучить уровни матрикс металлопротеиназы-8 и интерлейкина-1β в пробах гингивальной жидкости в течение первых трех месяцев ортодонтического лечения детей с помощью фиксированной техники.

МATERIAL И МЕТОДЫ: Прослежено 12 детей, леченных брекетами. Исследовано 48 проб гингивальной жидкости, собранных с одного репрезентативного зуба до постановки брекетов, через 24 ч., 1 нед., 3 мес. Обследовано количество гингивальной жидкости и уровни матрикс металлопротеиназы-8 и интерлейкина-1β.

Использованы стрип-ленты из фильтровальной бумаги для собирания гингивальной жидкости. После элюирования сделан количественный анализ биомаркеров через твердофазный иммуноэнзимный метод ELISA.

РЕЗУЛЬТАТЫ: Полученные результаты показывают легкое снижение уровней обоих маркеров по сравнению с исходными стоимостями и показывают повышение к третьему месяцу от начала ортодонтического лечения. Подобная тенденция наблюдается и в дебите гингивальной жидкости.

ЗАКЛЮЧЕНИЕ: Количественное исследование матрикс металлопротеиназы-8 и интерлейкина-1β в пробах гингивальной жидкости представляет собой возможный неинвазивный метод получения информации о процессах ремоделирования периодONTALного пространства в ходе ортодонтического лечения, благодаря чему процесс можно контролировать и ограничивать во физиологических границах.