ORIGINAL ARTICLE

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# Nitric oxide as prediction factor of gingival inflammation in orthodontic patients

## Azot oksid kao prediktivni faktor inflamacije gingive kod ortodontskih bolesnika

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### Abstract

**Background/Aim.** Nowadays therapy with fixed orthodontic appliances is widely spread, having in mind positive effects it has in malocclusion treatments The side effect is increased gingivial inflammation in treated patients. The aims of this research are to estimate the inflammatory condition of gingiva in the first 6 months of orthodontic therapy on the basis of clinical parameters of sulcus bleeding index, plaque index, gingival crevicular fluid and salivary nitric oxide concentration, and to investigate role of nitric oxide as predicting factor of gingival inflammation in orthodontic patients. **Methods.** In this study, 30 patients of the Dental Clinic treated with fixed orthodontic appliances (11 males and 19 females), aged 15–22 years, were examined. Clinical parameters were evaluated and gingival crevicular fluid and saliva were collected, before the beginning of orthodontics therapy, and then, three and six months after it. **Results.** The approximate values of clinical parameters, gingival crevicular fluid and salivary nitric oxide concentration progressively increased. Low statistical significance of correlations among gingival crevicular fluid and salivary nitric oxide concentration and the measured clinical parameters were found. There is a statistically significant correlation between gingival crevicular fluid and salivary nitric oxide concentration. **Conclusion.** According to the obtained results, we can conclude that gingival crevicular fluid and saliva are reliable mediums for monitoring of the gingival inflammatory condition. More studies are needed to investigate a potential role of nitric oxide as predicting factor of gingival inflammation in orthodontic patients.

**Key words:**
**orthodontics, corrective; nitric oxide; inflammation; gingival crevicular fluid; saliva.**

### Apstrakt

**Uvod/Cilj.** Ortodontska terapije fiksnim aparatima je široko rasprostranjena u terapiji malokluzija. Jedna od negativnih strana ove terapije je pojava zapaljenja gingive tretiranih bolesnika. Cilj rada bio je procena stanja zdravlja gingive u prvih šest meseci ortodontske terapije na osnovu vrednosti kliničkih parametara krvarenja gingive, plak indeksa, kao i koncentracije azot monoksida u pljuvačci i sulkusnoj tečnosti. Drugi cilj bio je utvrđivanje stepena korelacije koncentracija azot monoksida u pljuvačci i sulkusnoj tečnosti u toku prvih šest meseci terapije. **Metode.** Studijom je bilo obuhvaćeno 30 bolesnika Klinike za stomatologiju lečenih fiksnim ortodontskim aparatima (11 muškog, 19 ženskog pola), starosti 15–22 godine. Određivani su parametri, a pljuvačka i sulkusna tečnost su sakupljani pre početka, kao i tri i šest meseci posle početka terapije. **Rezultati.** Utvrđen je statistički značajan porast vrednosti kliničkih parametara i koncentracije azot monoksida u toku prvih šest meseci ortodontske terapije. Nađeni su nizak nivo statističke značajnosti korelacije merenih kliničkih parametara i koncentracije azot monoksida u pljuvačci i sulkusnoj tečnosti, kao i statistički značajna korelacija koncentracija azot monoksida u pljuvačci i sulkusnoj tečnosti u toku prvih šest meseci terapije. **Zaključak.** I gingivalna sulkusna tečnost i pljuvačka su pouzdani medijumi za praćenje stanja zdravlja gingive kod ortodontskih pacijenata. Potrebno je sprovesti još studija koje bi rasvetlile mogućnost korišćenja azot monoksida kao faktora za praćenje stanja zdravlja gingive kod ortodontskih bolesnika.

**Ključne reči:**
**ortodoncija, korektivna; zapaljenje; gingivalna sulkusna tečnost; pljuvačka.**

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## Introduction

Nowadays orthodontic therapy with fixed orthodontic appliances is widely spread, having in mind positive effects it has in malocclusion treatments [1]. The side effect of therapy is that the constant presence of orthodontic brackets in mouth cavity prevents adequate oral hygiene, increases the number of retaining locations where dental plaque can be acumulated and provokes mechanical irritations of oral mucosa. Numerous studies have confirmed the presence and increased gingivial inflammation within the patients having the orthodontic braces [2–6].

Periodontal disease is an inflammatory process of the periodontal tissues (gingiva, alveolar bone, cement, periodontal fibres), affecting single or multiple locations [7].

Progression of periodontal disease is at large scale conditioned by a patient's individual characteristics [8].

Microbiological basis of periodontal diseases was verified long ago. The microorganisms of the oral biofilm operate in two ways: they directly aggravate the tissue of the host and provoke the release of numerous biological mediators which could lead to the tissue destruction. The mediators being a part of organism's reaction to bacterial infection and, as such, leading to the tissue decay are: proteinases, cytokines and prostaglandins [9].

Traditional diagnostical clinical methods, such as assessing the pockets' depth, bleeding on probing, determing the clinical loss of the ephitelial lining, plaque index and radiography are more useful for the determination of the presence and consequences of a periodontal disease rather than its activity. There is a need to develop some new diagnostical tests that might indicate the presence of active disease, its progression and effects it has on the applied therapy.

Gingival crevicular fluid (GCF) represents an exudate found in gingival sulcus. It consists of serum, although its composition may vary depending on the neighbouring gingival tissue and bacterial presence, so that it may contain immunoglobulins, toxins, cells, microorganisms and numerous enzymes [10]. GCF has recently been used as the medium for measuring and quantifying various molecules and bacteria presence in the mouth cavity as well as in the periodontal ligament space [11–13]. Markers of the bone remodelation in orthodontic patients may also be detected [14].

Nitric oxide (NO) is a free radical with many biological functions. It is an intercellular signaling molecule involved in many physiological functions in organism: regulation of vascular tonus, intestinal motility, aggregation and adhesion of thrombocytes, the formation and destruction of bones, numerous immunological functions, apoptosis, and neurotransmission. It is a highly-reactive molecule, easily diffusing through a cell membrane [15]. Endothelial and neuronal cells produce NO constitutively, but it can be also produced by macrophages and other inflammatory cells in pathological conditions. The most important stimuli for NO synthesis are bacterial products. In inflammatory processes of periodontium, the positive effects of NO are related to bacteria destruction, while negative effects involve the damage of the tissue through the mechanisms of oxidation, nitric reactions, enzyme inhibitions, DNA distraction, metalloproteinase activation. As other free radicals, NO participates in neutrophile procolagenase activation, in the supression of protheoglycans and collagen synthesis, thus contributing to gingival damage advancement. The findings from the different studies suggest that NO levels are increased in GCF and serum in subjects with periodontal disease, compared to the healthy controls. In the research conducted on 90 persons [16], the authors concluded that the increase in GCF NO levels was directly proportional to the severity of periodontal disease. Skaleric et al. [17] examined GCF of 18 diabetic patients and their results showed the increased GCF NO level in patients with more severe gingival inflammation. In the literature there are no data about GCF NO as the marker of gingival inflammatory condition in patients with fixed orthodontic appliances.

The aim of this study was to estimate the inflammatory condition of gingiva in the first six months of orthodontic therapy on the basis of clinical parameters of Sulcus Bleeding Index (SBI), Plaque Index (PI), GCF and salivary NO production, so that the degree of correlation between clinical parameters and the NO production can be established with a possibility to show the periodontic inflammatory condition.

## Methods

### *Study population*

This study included 30 orthodontic patients (11 males and 19 females) of the Dental Clinic, aged 15–22 years, treated with fixed orthodontic appliances.

The selected patients fulfilled the next criteria: 1) need for non extraction orthodontic treatment with fixed appliances, 2) absence of approximal caries or approximal fillings on permanent molars and second premolars, 3) good systemic health of patient (absence of chronic diseases history), 4) absence of use of anti-inflammatory and antibiotic therapy three months before the beginning of the treatment.

Exclusion terms were: 1) mouth breathing patients, 2) patients with severe crowding 3) patients with dentofacial deformities, 4) treatment by dental hygienist any time during the first six months of therapy, 5) use of anti-inflammatory and antibiotic therapy during first six months of the orthodontic treatment, 6) smoking habits.

Informed consent and approval document for the participation in the study were signed by the patients or their parents if patients were younger than 18 years. The study protocol was approved by the Ethical Committee No 01-890-6.

### *Clinical procedure*

Specialist of orthodontics was evaluating clinical parameters (PI), (SBI) and collecting GCF and saliva.

At the beginning of the treatment, the patients were given detailed instructions about oral hygiene procedures that they should follow out during orthodontic treatment. The patients were not allowed to use anti plaque substances during first six months of treatment. During first six months of the therapy only nivelation of dental arches was done, using round NiTi arch wi-

res 0.12, 0.14 and 0.16 at the end. The patients using antibiotics and antiinflammatory drugs during first six months of therapy were excluded from the study.

Supra- and subgingival ultrasound cleaning was conducted in all patients two weeks before the beginning of orthodontic treatment.

All patients from the study were treated using fixed orthodontic appliances, following basic principles of technique of straight arch. Control checkups were conducted precisely in one month periods.

*Evaluation of clinical parameters*

Clinical parameters of gingival inflammation Silness-Löe PI and Muhlemann-Son SBI [18] were evaluated using periodontal probe and respecting World Health Organization (WHO) criteria, before, three and six months after the beginning of the orthodontics therapy during regular orthodontic controls. We selected particular time points in the study, since we found these time periods minimal for clear expression of gingival inflammation in orthodontic patients.

Before starting investigation, the necessary calibrations were performed to provide validity of the results. To test intra-examiner agreement, examiner re-measured PI and SBI in 30 persons two weeks after initial measurements.

Kappa statistics was used to evaluate the consistency of intra-examiner agreement.

*GCF collection*

GCF was collected using paper strips before, three and six months after the beginning of orthodontic therapy. The teeth to be sampled were isolated with cotton rolls in order to avoid saliva contamination. Supragingival dental plaque was removed. The paper strip was inserted into the crevice on vestibular surface of first permanent molars, second premolars, canines and central incisors until mild resistance is felt and it was left there for 30 sec. It means that eight paper strips from every patient were collected at the end. Paper strips contaminated with blood because of gingival irritation were excluded from study. All collected samples of GCF were stored in sterile eppendorfs at −80 ºC until the next step in their elaboration at the Institute of Biochemistry.

*Saliva collection*

After making the subjects rinse their mouths thoroughly with water, salivary samples were collected in sterile containers by instructing them to allow saliva to collect naturally in mouth and to expectorate it into the containers. All collected samples of saliva were stored at -80 $^{o}$C until the next step in their elaboration at the Institute of Biochemistry.

*Biochemical analyses*

After deproteinization, the production of NO was evaluated by measuring $NO_2^- + NO_3^-$ concentrations. Nitrates were transformed into nitrites by cadmium reduction, before the measuring of total $NO_2^- + NO_3^-$ concentration [19]. Nitrites were assayed directly spectrophotometrically at 543 nm, using the colorimetric method of Griess. Salivary and GCF NO concentrations are rendered by proteins.

*Statistical analysis*

The results from the study are presented in tables and figures. The values of examined parameters are represented with mean values (r), standard deviations (SD), 95% confidence intervals (95% CI), medians (MD) and interquartile ranges (IQR). The distributions of the continuous variables were assessed for normality by Shapiro-Wilk test. A paired *t*-test was used in case of two related observations with a normal distribution, and Wilcoxon Signed-Ranks if a distribution of data was not normal. Depending on the distribution of normality Pearson (r), or Spearman (ρ) correlation coefficients were used to analyze associations between continuous variables. Statistical data analysis was done with the SPSS software package (Version 18) where significance level was $p < 0.05$.

**Results**

The kappa values of the intra-examiner reproducibility for PI and SBI were 0.82 and 0.86, respectively.

When these values were analysed, almost perfect agreement was obtained for both PI and SBI.

There was a statistically significant increase of values of clinical parameters (PI and SBI) three and six months after the beginning of the orthodontic therapy in comparison to the values before beginning of the therapy, ($p < 0.001$). There was also a statistically significant increase of GCF and salivary NO concentration three and six months after the beginning of therapy in comparison to the values before the beginning of the therapy ($p < 0.001$) (Figures 1–4).

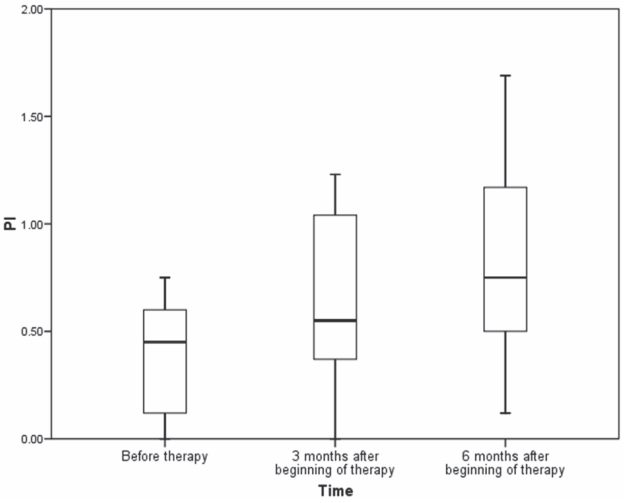


**Fig. 1 – Box plots with medians and interquartile ranges of Plaque Index (PI) before and three and six months after the beginning of the orthodontic therapy.**

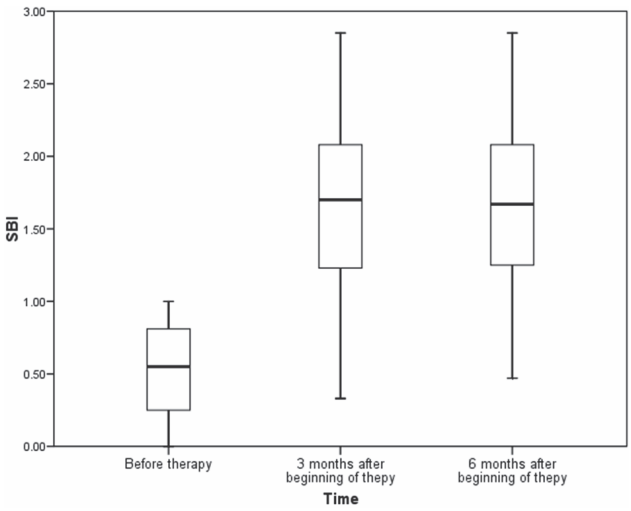


**Fig. 2 – Box plots with medians and interquartile ranges of Sulcus Bleeding Index (SBI) before, and three and six months after the beginning of the orthodontic therapy.**

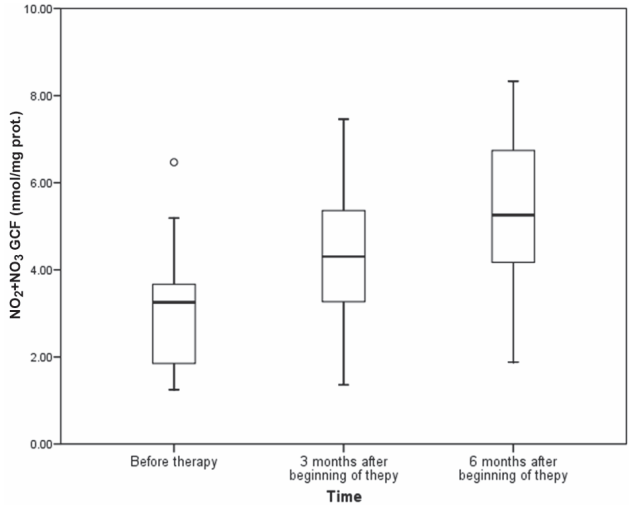


**Fig. 3 – Box plots with medians and interquartile ranges of gingival crevicular fluid (GCF) $NO_2^-$ + $NO_3^-$ concentration before, 'and three and six months after the beginning of the orthodontic therapy.**
**$NO_2^-$ – nitrite; $NO_3^-$ – nitrate.**

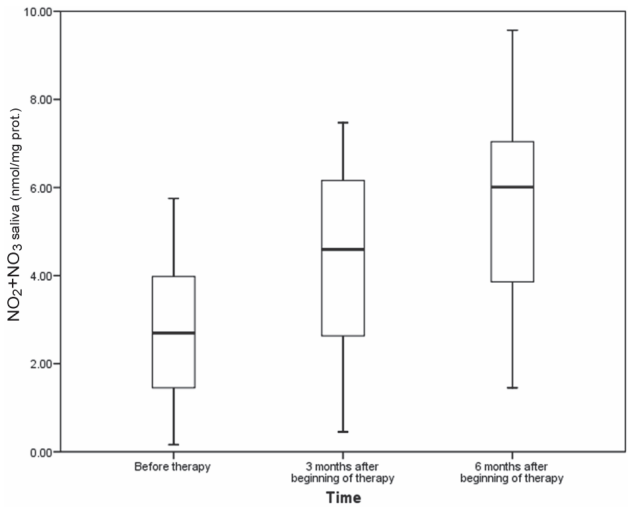


**Fig. 4 – Box plots with medians and interquartile ranges of salivary $NO_2^-$ + $NO_3^-$ concentration before, and three and six months after the beginning of the orthodontic therapy.**
**$NO_2^-$ – nitrite; $NO_3^-$ – nitrate.**

There was a positive statistically significant correlation between clinical parameters of the gingiva (PI and SBI) in the period of first three months of the orthodontic therapy ($p < 0.05$) and even bigger significance after six months of the therapy ($p < 0.01$) (Table 1).

There was a positive statistically significant correlation between GCF and salivary $NO_2^-$ + $NO_3^-$ concentration before beginning of the orthodontic therapy ($p < 0.01$), 3 months after the beginning of the therapy ($p < 0.05$) and even bigger significance six months after the beginning of the therapy ($p < 0.01$) (Table 1).

There was a negative statistically insignificant correlation between GCF and salivary $NO_2^-$ + $NO_3^-$ concentrations with both clinical parameters of gingiva PI and SBI except between SBI and salivary $NO_2^-$ + $NO_3^-$ concentration before the therapy and between PI and salivary $NO_2^-$ + $NO_3^-$ concentration six months after the beginning of the therapy ($p < 0.05$) (Table 1).

**Table 1**

**Correlations of examined variables during orthodontic therapy**

| Variable | Before therapy | 3 months after beginning of therapy | 6 months after beginning of therapy |
|---|---|---|---|
| PI and SBI | 0.46* | 0.41* | 0.46** |
| PI and $NO_2^-$ + $NO_3^-$ GCF | -0.20 | -0.17 | -0.10 |
| PI and $NO_2$+$NO_3$ saliva | -0.19 | -0.27 | -0.41* |
| SBI and $NO_2$+$NO_3$ GCF | -0.08 | 0.15 | 0.05 |
| SBI and $NO_2$+$NO_3$ saliva | -0.42* | -0.30 | -0.24 |
| $NO_2$+$NO_3$ GCF and $NO_2$+$NO_3$ saliva | 0.53** | 0.43* | 0.71*** |

***$p < 0.05$; ****$p < 0.01$, *****$p < 0.001$.**
**PI – Plaque Index; SBI – Sulcus Bleeding Index; $NO_2^-$ – nitrite; $NO_3^-$ – nitrate; GCF – gingival crevicular fluid.**

**Table 2**

**Changes of PI, SBI, GCF and salivary $NO_2^-$ + $NO_3^-$ concentration during orthodontic therapy (changes *vs* values before therapy)**

| Changes of variable | 3 months after the beginning of the therapy | 6 months after the beginning of the therapy |
|---|---|---|
| PI | 0.27 ± 0.26 (0.17–0.36)<br>0.28 (0.00–0.49) | 0.42 ± 0.35 (0.29–0.55)***<br>0.39 (0.00–0.62) |
| SBI | 1.04 ± 0.52 (0.85–1.24)<br>1.07 (0.54–1.50) | 1.17 ± 0.53 (0.97–1.36)*<br>1.21 (0.74–1.46) |
| $NO_2^-$ + $NO_3^-$ GCF (nmoL/mg prot.) | 1.34 ± 0.99 (0.97–1.71)<br>1.06 (0.3 –1.94) | 2.20 ± 1.22 (1.74–2.65)**<br>1.81 (1.51–3.41 |
| $NO_2^-$ + $NO_3^-$ saliva (nmoL/mg prot.) | 1.60 ± 1.08 (1.20–2.00)<br>1.16 (0.75–2.53) | 2.89 ± 1.50 (2.33–3.45)***<br>2.60 (1.83–3.98) |

**Data are given as means ± standard deviation (SD) (95% confidence interval – CI), medians (Md) interquartile ranges (IQR).**
***p* < 0.05, ***p* < 0.01, ****p* < 0.001.**
**For abbreviations see under Table 1.**

**Table 3**

**Correlations of PI, SBI , GCF and salivary $NO_2^-$ + $NO_3^-$ concentration changes during the orthodontic therapy**

| Variable | 3 months after the beginning of the therapy | 6 months after the beginning of the therapy |
|---|---|---|
| PI and SBI | 0.27 | 0.31 |
| PI and $NO_2^-$ + $NO_3^-$ GCF | 0.02 | 0.11 |
| PI and $NO_2^-$ + $NO_3^-$ saliva | –0.19 | 0.03 |
| SBI and $NO_2^-$ + $NO_3^-$ GCF | 0.13 | 0.03 |
| SBI and $NO_2^-$ + $NO_3^-$ saliva | –0.25 | –0.04 |
| $NO_2^-$ + $NO_3^-$ GCF and $NO_2^-$ + $NO_3^-$ saliva | 0.31 | 0.59*** |

*****p < 0.001.**
**For abbreviations see under Table 1.**

Changes of initial values of all examined parameters six months after the beginning of the therapy in comparison to those three months after the beginning of the therapy were significantly higher, SBI ($p < 0.05$), GCF $NO_2^-$ + $NO_3^-$ ($p < 0.01$), PI and salivary $NO_2^-$ + $NO_3^-$ ($p < 0.001$) (Table 2).

There was a positive, but statistically insignificant correlation of changes between PI and SBI during first three and six months of the therapy.

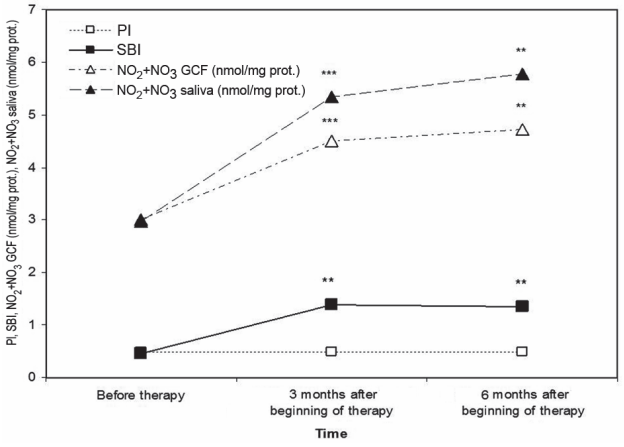


**Fig. 5 – Medians of PI, SBI, GCF and salivary $NO_2^-$ + $NO_3^-$ concentration in patients with constant values of PI before, three and six months after the beginning of the orthodontic therapy (**$p < 0.01$, ***$p < 0.001$). For abbreviations see under Table 1.**

There was also a positive but statistically insignificant correlation of changes between CGF and salivary $NO_2^-$ + $NO_3^-$ concentration after three months of the therapy, while it became strong and statistically significant after six months of the therapy ($p < 0.001$) (Table 3).

Eight patients of the examined group did not have changes in PI values during the first six months of the therapy, but there was a statistically significant increase of values of the clinical parameter SBI, three and six months after the beginning of the therapy compared to the values before the beginning of the therapy ($p < 0.01$). In the same group, there was also a statistically significant increase of GCF and salivary $NO_2^-$ + $NO_3^-$ concentration three ($p < 0.001$) and six months after the beginning of the therapy ($p < 0.01$) (Figure 5).

## Discussion

In our research, the important findings are statistically significant increase of the values of PI and SBI, three and six months after the beginning of the therapy compared to those before the therapy. The results given indicate the presence of the gingival inflammation and worsening of the inflammatory processes within the first six months of the therapy. All these results are in agreement with the results of numerous studies [3–6, 20].

Although periodontal disease can have difficult diagnosis and treatment, this article evaluates gingivitis and it is

important to note that gingivitis is easily diagnosed through clinical observation.

Out of 30 patients, eight of them did not have any changes in PI after three and six months of the therapy, when the results were compared to those obtained before the therapy was applied. Such results suggest adequate oral hygiene in spite of the circumstances that include the orthodontic braces in mouth cavity. During the therapy, there was, however, the increase of the values of SBI, GCF and salivary NO production compared to those before the therapy. These results indicate that among the patients having orthodontic braces, gingival inflammation, apart from plaque presence, is influenced by some other factors, such as bonding, bracket gluing, and mechanical irritation of gingiva. Corbacho de Melo et al. [5] pointed the fact that positioning of the bracket edge below the gingival margin provoked gingival inflammation to a large extent.

There are very few researchers who examined the role of NO in the process of a bone remodeling during the initial phases of orthodontic teeth movement [21]. They suggest that NO is involved in the regulation of the second messenger system formation, in the regulation of osteoblasts and osteoclasts functions and the blood flow in the pulp. It has been determined that NO increases microvascular permeability and, as a such, can acquire a crucial role in the first stages of bone remodeling due to the fact that the blood vessels monocytes become the basis of a bone remodeling later during the orthodontic therapy [22]. Being familiar with the role of NO in the process of bone remodeling during the orthodontic teeth movements is very important for the interpretation of our results. Samples of GCF in our study were always taken one month after the previous control and the prospective application of orthodontic force and always before taking the next step in the therapy. In this way, we have tried to reduce the influence of the orthodontic force on NO production and make it, as much as possible, a measure of gingival inflammation caused by the presence of the orthodontic braces in the mouth cavity.

Our results point out the statistically significant increase of GCF and salivary $NO_2^- + NO_3^-$ concentration three and six months after the beginning of the orthodontic therapy. Changes in NO production during this period, as our research shows, may indicate tendency of gingiva inflammation increase among some patients. Still, there is a low correlation between clinical parameters of gingival condition (PI and SBI) and GCF and salivary $NO_2^- + NO_3^-$ concentrations during the-six-month-long-therapy. This fact indicates that GCF NO production in the orthodontic patients might be influenced not only by gingival inflammation but also by some other factors related to the nature of orthodontic treatment and the process of bone remodeling it is followed by.

NO is generated by the nitric oxide synthase (NOS) enzymes from the oxygen and the amino acid L-arginine. There are three isoforms of NOS: a neuronal form (nNOS), an endothelial form (eNOS) and an inducible form (iNOS). High levels of eNOS are detected in the endothelial cells of the blood vessels. NO is important factor responsible for the relaxation of the blood vessels smooth muscles in compressed areas during tooth movement [23, 24]. Periodontal hyperaemia is the initial phase that leads to complex processes of bone remodeling during orthodontic movement of teeth.

D'Attillio et al. [25] examined eNOS and iNOS levels of gingival tissue in patients treated with fixed orthodontic appliances. In their study, canine undergoing treatment for distal movement served as the test tooth whereas its contralateral canine was used as the control tooth. Two weeks after beginning of the therapy both eNOS and iNOS levels were significantly higher in gingiva of the test tooth than those of the control tooth. They concluded that gingival tissue, surrounding a moving teeth, did not undergo resorption, but was compressed and retracted. The role of gingival eNOS, iNOS and NO during the early phases of orthodontic treatment in humans is of significant importance, as obvious.

Two major cell types responsible for bone remodeling are osteoclasts, which resorb bone, and osteoblasts, which form new bone. There are many studies that indicate NO role in promoting osteoclasts differentiation and bone resorption [26, 27].

During orthodontic tooth movement, there is also an increase in number and activity of macrophage-like cells in resorptive areas or periodontal tissue undergoing more intensive mechanical stress [27]. Macrophages remove necrotic periodontal tissue and during the process of cell interaction, they release NO [28]. Gaspirc et al. [29] showed detectable levels of iNOS in macrophages of gingival tissue.

Briefly, GCF NO concentration during orthodontic tooth movements depends on the activity of several cell types in bone, periodontium and gingiva. Beside its main role in initial phase of bone remodeling, there are different complicated processes that could influence GCF NO concentration even one month after starting with orthodontic force. That could explain the lack of statistically significant correlations between GCF, salivary NO production and clinical parameters of gingival inflammation in our study. On the other hand, the clinical parameters are more useful in the determination of the presence and consequences of periodontal disease rather than its activity, so missing of the correlation among the PI, SBI and GCF NO production in our study can be expected, having in mind that many authors have suggested NO as a marker of inflammation activity.

The question that arises is whether GCF and saliva can be equally reliable parameters for monitoring of periodontal conditions. Saliva sampling, compared to that of GCF one, is a pretty simple procedure and thus much larger quantities of it can be available. Certain authors consider GCF to be a more reliable source for identifying and monitoring periodontal diseases. The reason lies in the fact that GFC is solely under the influence of the neighbouring periodontal tissues. On the other hand, saliva is primarily formed by the secretion of salivary glands that are also responsible for NO production. One more reason that makes saliva and its diagnostic potential a less reliable parameter in identifying the periodontal disease is that it can easily reflect both systemic inflammatory and infectious conditions [10].

The results we obtained indicate statistically significant increase of NO production in saliva and GCF three and six months after the beginning of the orthodontic treatment. It is also proven

that there is a statistically significant correlation between the values of GCF and salivary NO production before, three and six months after the beginning of the orthodontic treatment.

There is a positive but at the same time statistically insignificant correlation in the change of salivary and GCF $NO_2^- + NO_3^-$ concentrations that occurred three months after the treatment started. However, there is a strong statistically significant correlation in the changes of the salivary and GCF $NO_2^- + NO_3^-$ concentrations that occurred six months after the beginning of the orthodontic treatment. All these facts lead to a conclusion that GCF and saliva can be used as a reliable medium for periodontal conditions monitoring. The given results coincide with the results achieved by Topcu et al. [30]. On the other hand, Poorsattar Bejeh Mir et al. [31] concluded that detecting NO biomarker and its end metabolites in saliva was of more value to assess the periodontal health when comparing to GCF.

## Conclusion

According to the measurements of the values of the clinical parameters, PI and SBI, related to the gingival inflammatory status as well as the measurements of GCF and salivary NO concentration, it can be concluded that there is the increase of gingival inflammation during the first six months of the orthodontic therapy with fixed appliances. In spite of the fact that our results indicate a statistically significant increase of GCF and salivary NO concentration during the first six months of orthodontic therapy, one should be very cautious because there is a low correlation with the clinical parameters.

According to the results obtained in this study, we can suggest that both GCF and saliva are reliable mediums for monitoring the gingival inflammatory condition.

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