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Modern methods of salivary nitric oxide rapid detection in different types of orthodontic appliances

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Abstract

The treatment of dento-maxillary abnormalities is made with different types of appliances (fixed or removable) and usually involves high intensity forces that will, more often than not, result in an inflammatory (aseptic and transient) response at the periodontal level. This inflammatory response is accompanied by an increase in free radical synthesis, secondary to oxidative stress. In orthodontic field, Nitric oxide (a biomarker of oxidative stress), is considered a regulator of the bone response to mechanical stress and it mediates adaptive bone formation. The aim of this study is to investigate the effect of different orthodontic appliances on nitric oxide levels. Three groups of subjects have been tested: one group of ten patients with metallic fixed appliances, one group of ten patients with aligners, and a control group consisting of five subjects. Immunochromatography method was used and salivary samples were obtained from all individuals before treatment, during the 1st and the 2nd month of treatment. The groups were compared statistically by means of Student T tests and ANOVA and showed statistically significant difference. In conclusion, these result show that different types of orthodontic appliances increase the synthesis of nitric oxide in various levels.

Keywords: oxidative stress, nitric oxide, orthodontic aligners, fixed appliances.

1. Introduction

Orthodontics (as a branch of dental medicine), occupies an increasingly important role in people's lives. The increasing incidence of global dento-maxillary anomalies, generates and demands ongoing research for continuously improving orthodontic treatment. A favorable outcome confers an aesthetic, social and, above all, functional status of major importance.

Because of this, the modern dental appliances are smaller and less visible. The traditional metal brackets are currently significantly reduced in size, the lingual technique and esthetic brackets are more frequently used and the last type of dental appliance (aligner) is well promoted and asked for by patients.

The imbalance between reactive oxygen species (ROS) formation and enzymatic and non-enzymatic antioxidants give rise to a phenomenon known as oxidative stress. Several cellular structures (membranes, lipids, proteins, lipoproteins and deoxyribonucleic acid) can be affected by this harmful process and can trigger apoptosis leading to cellular death (M. Portelli et al [1]). Oxidative stress can be incriminated in generating several diseases (acute, chronic and degenerative) and speeding up body aging processes. It is scientifically proven that it can play an important role in cancer development, atherosclerosis, ischemia, hypertension, cardiomyopathy, cardiac hypertrophy, congestive heart failure, neurological diseases, multiple sclerosis, depression, memory loss (neuron loss and progression to dementia), asthma, chronic obstructive pulmonary disease, Type 2 Diabetes, Rheumatoid arthritis, glomerulus and tubule-interstitial nephritis, renal failure, proteinuria, uremia, delayed sexual maturation, and puberty onset (G. Pizzino et al [2], D. Timofte et al [3]). In oral disease, the oxidative stress is correlated with progression to periodontitis, oral lichen planus, oral cancer and myofascial pain (J. Kumar et al [4], D. Basi et al [5]).

Saliva could be considered the first line of defense against oxidative stress (because its' rich antioxidants content). Due to its' composition and functions, saliva may play a significant role in the control and / or modulation of oxidative damage in the oral cavity (M. Greabu et al [6]).

There is a relatively large number of studies whose results support the association between oral diseases and oxidative stress markers altered in saliva. In healthy patients there are fluctuations, depending on the time of collection and sex (I.Z Alajbeg et al [7], I. Lettrichová [8]). Unstimulated saliva is most appropriate for diagnostic studies because of its noninvasive nature, rapid stimulation, and ease of sampling. A variety of bone-remodelling biomarkers have been identified amongst its' components (N.H Ford [9]). In the dental field, it helps determine the prevalence of dental caries, the diagnosis and prognosis of periodontal disease (it is a reliable environment for monitoring the inflammatory gingival condition) and oral cancers (due to HIV) or Herpesvirus and Sjögren's syndrome (C-Z Zhang et al [10], P. Janošević et al [11]).

NO (nitric oxide) is a free radical and an intercellular signaling molecule involved in 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 produced by endothelial and neuronal cells, macrophages and other inflammatory cells in pathological conditions but the most important stimuli for NO synthesis are bacterial products. During inflammatory processes of periodontium, NO can have positive effects like bacteria destruction, while negative effects involve damage to tissues (mechanisms of oxidation, nitric reactions, enzyme inhibitions, DNA distraction, metalloproteinase activation). As other free radicals, NO contributes to gingival damage advancement (because of his role in neutrophils procollagenase activation, as well as suppression of proteoglycans and collagen synthesis). During tooth movement it is an important factor, responsible for the relaxation of the blood vessels in compressed areas (the first phase of the orthodontic movement of teeth is periodontal hyperemia which leads to a complex processes of bone remodeling) (P. Janošević et al [11]).

To our knowledge, a comparison between metallic braces and aligners has not been previously described in literature. The aim of this study, therefore, is to investigate the effects of conventional braces in comparison to the removable appliance (aligners) regarding nitric oxide level as a biomarker of oxidative stress.

2. Materials and Methods

Subjects

The study group consisted in 25 subjects divided in 3 subgroups: the control group (A) formed by 3 females and 2 males, aged 22 to 30 (mean 27.4 years), the group with metallic braces (B) consisted in 6 females and 4 males aged 16 to 30 (mean 22.9 years) and the group wearing aligners (C) was formed by 8 females and 2 males aged 18 to 30 (mean 25.4 years). Inclusion criteria for this study were patients of both genders who were in the age group of 16-30 years (after the growth spurt), with no systemic disease, no antibiotic treatment for the previous six months, no anti-inflammatories one month previous to orthodontic treatment, no oral contraceptives, non-pregnant, with no increased carious activity or active cavities at the start of treatment, with permanent dentition, non-smokers, with no personal history of drug use, candidate for fixed orthodontic treatment with: Skeletal Class I, with no need for extractions, excessive expansion, anchorages auxiliary elements (with mild/moderate crowding exhibiting no greater than 6 mm of space discrepancy per arch), with no history of trauma, bruxism or parafunctions, with no signs or symptoms of clinical or radiological periodontal disease, without large dental restorations (fixed or mobile), without palatinal/ lip cleft or anodontia, capable of good collaboration and good oral hygiene.

On the day the samples were taken, in order to avoid an interaction between any substance contained in the toothpaste and the molecules to be examined, the patients were asked to brush their teeth without toothpaste and to refrain from eating and drinking for at least 30 minutes prior to the visit. All subjects were encouraged to religiously maintain their oral hygiene. Approval from the Ethics Committee of „Ovidius" University Constanta was granted in accordance with the principles of Declaration of Helsinki and informed consent was obtained prior to the sample collection.

Types of orthodontic appliances

The conventional metallic braces used in this study were made from high-strength stainless steel (Micro Sprint, Forestadent, Pforzheim, Germany) and the archwires were size 0.12 Ni-Ti (LTS form, American Orthodontics, USA). The aligners were made from Polyethylenterephthalat-Glycol Copolyester (PET-G) (Clear Aligner, Scheu-Dental GmbH, Germany) which fulfills the necessary biocompatibility conditions for medical products. The composite and primer used were purchased from Ormco, USA (Enlight Light Cure Adhesive™ and Ortho Solo™). After the appliance was fixed in the subject's mouth, anti-inflammatory treatment was strongly discouraged (they were instructed to only use acetaminophen 500 mg in case of marked discomfort).

Biomarker assessment using strips

The Nitric Oxide (NO) assessment in saliva, was made using an immunochromatographic method in accordance to the package inserts (Nitric Oxide Saliva Test Strips, Berkeley, USA). The strip was put directly on the tongue and after10 seconds the result was compared with the color chart provided by the strip manufacturer. The samples were taken before the appliance was in place (T0), after one month (T1) and after two months (T2).

Statistical Analysis

Analysis of the data was performed using SPSS for Win. Ver. 20.00 (SPSS Inc., IL, USA) software packages. The ANOVA single factor test and the Student T-test were employed to verify and find statistical differences between the test groups. A value of <0.05 was considered as statistically significant.

3. Results and discussion

The mean age of a total of 25 patients, including 6 males and 19 females with ages ranging between 16 and 30 years, was 25.2 years. Among patients treated with fixed orthodontic treatment and aligners, salivary tests were made prior to orthodontic treatment (T0), at the first (T1) and second month (T2). For the control group, the same analytical times were used, and nitric oxide did not vary at any point. The distribution of Nitric Oxide is showed in Table 1.

Table 1. Nitric oxide score distribution in different stages of the treatment

	Results (µm) / Time	DEPLETED (20 µm)	LOW (110 µm)	THRESHOLD (220 µm)	TARGET (435 µm)	HIGH (870 µm)
CONTROL (A)	T0	4	0	1	0	0
	T1	4	0	1	0	0
	T2	4	0	1	0	0
METALLIC BRACES (B)	T0	5	5	0	0	0
	T1	2	0	8	0	0
	T2	2	4	4	0	0
ALIGNER (C)	T0	4	5	1	0	0
	T1	4	5	1	0	0
	T2	5	5	0	0	0

* Time (T0, T1, T2) – at the beginning of the treatment (T0), at one month (30 days) (T1) and after two months (60 days) (T2)
Groups (control (A), metallic braces (B), aligner (C)) – Control- 5 normal patients that don't require orthodontic treatment;metallic braces – 10 patients with fixed orthodontic appliancealigner – 10 patients wearing aligners
Results (depleted, low, threshold, target, high) – in µm; results according with the color chart provided by the strip manufacturer

Between metallic braces and control in T1 there was a statistically significant difference ($p<0.05$). In T2 an increase was also noticed, but lower that in T1. Between the aligner and control there were no differences in T1 but a slight decrease in NO levels could be noticed in T2. Comparing the group of aligners with the metallic one there was a significant difference both in T1 and T2 ($P<0.05$) (Figure 1). The results of ANOVA test to determine any significant difference between all groups shows that there was a statistically significant difference in T1 ($P<0.05$).

Discussion

Orthodontic treatment is an important tool in facial esthetics, dental esthetics (most coveted by patients), functional occlusion and in periodontal health. The evolution of orthodontic appliances was from metallic bands to braces (on the buccal or lingual part, esthetic or metallic, with self-ligation or conventional) and after that, to something even less visible, like aligners, which are increasingly utilized and required.

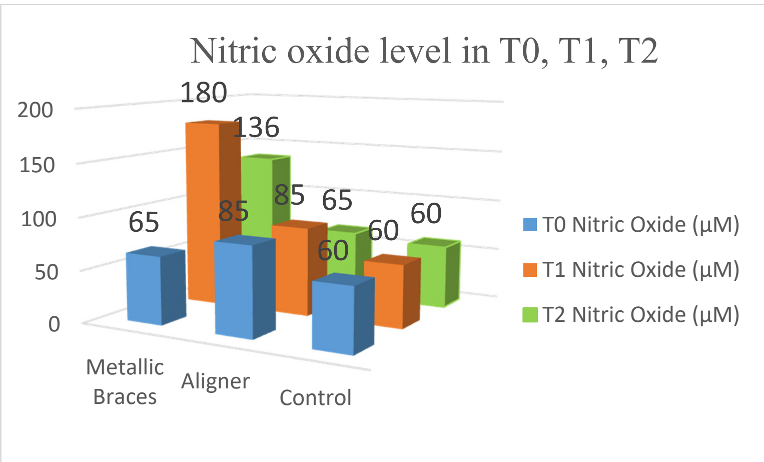

Figure 1. Compared nitric oxide levels in all groups

Tissues produce free radicals like reactive oxygen or nitrogen species (RONS) during physiological processes, which can act as intercellular or intracellular messengers. The most frequently generated are hydroxyl radicals, hydrogen peroxide, nitric oxide, peroxides, peroxynitrite, singlet oxygen, and superoxides. The harmful effects of free radicals are counterbalanced by antioxidant mechanisms. An alteration in this balance, in favor of free radicals, generates oxidative tissue damage associated with oxidative stress (N. Jha et al [12], S.S Özcan Atug et al [13]).

Nitric Oxide (NO) - is considered a regulator of bone response to mechanical stress. It mediates adaptive bone formation: the increased level reduces osteoclast activity and vice versa (S.D Tan et al [14]). A. Modi et al [15] compared rapid salivary tests for nitric oxide detection (Berkley and Neogenesis) with lab measurements and the result was that strips are capable of providing a reasonable surrogate to specific tests. There are very few researchers who examined the level and role of NO during orthodontic teeth movement. *Our study was the first one* to compare the conventional treatment with aligners (and the first one using immunochromatographic method of rapid detection of nitric oxide -as a biomarker of oxidative stress). Therefore we could not refer to literature results. The NO level for aligners did not change, on the contrary, showed a slight decrease, potentially as a result of oral hygiene improvement. When compared to metallic devices, a statistically significant difference was noticed.

After the application of fixed metal appliances, there was a significant increase in nitric oxide, especially during the first month. This result was in agreement with the study made by J.A Alarcon et al [16] and P. Janošević et al [11] and in contradiction with the results of S.S Özcan Atug et al [13]. J.A Alarcon et al [16] used colorimetric method and the level of nitric oxide was increased from the seventh day to the 30th day after the start of fixed orthodontic treatment (in adult women). P. Janošević et al [11] used spectophotometric method and the salivary NO concentration had a statistically significant increase in the first six months of orthodontic treatment but this result was poorly correlated with clinical parameters (clinical parameters of gingival inflammation were used). S.S Özcan Atug et al [13] used spectophotometric method and NO level remained within physiological limits and did not change in all samples at any stage of treatment.

4. Conclusion

a) In fixed orthodontic treatment, the materials used, the force and the hygiene may change the salivary Nitric oxide levels that *can be suggestive for oxidative damage*.

b) Fixed appliances compared with aligners are more invasive. Aligners do not change the level of salivary nitric oxide.

c) This simple method of detection can be a starting point for complex methods, more specific and sensitive of determining salivary nitric oxide levels.

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