Advances in Medical Sciences 62 (2017) 129–135

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original research article,

Orthodontic treatment modifies the oxidant–antioxidant balance in saliva of clinically healthy subjects

Piotr Buczko [a,*], Małgorzata Knaś [b], Monika Grycz [a], Izabela Szarmach [a], Anna Zalewska [c]

[a] Department of Orthodontics, Medical University of Bialystok, Bialystok, Poland
[b] Institute of Health Care Higher Vocational School, Suwalki, Poland
[c] Conservative Dentistry Department, Medical University of Bialystok, Bialystok, Poland

ARTICLE INFO

Article history:
Received 10 November 2015
Accepted 29 November 2016
Available online

Keywords:
Orthodontic appliances
Oxidative stress
Saliva nickel

ABSTRACT

Purpose: The aim of our study was to analyse salivary markers of oxidative stress and an antioxidant response in clinically healthy subjects with fixed orthodontic appliances.
Material/methods: 37 volunteers were included in the study. Unstimulated (UWS) and stimulated (SWS) whole saliva were analysed for oxidative and antioxidant status and nickel levels immediately before the insertion of the appliances, an one week after and twenty four weeks after the insertion of fixed appliances.
Results: A significant increase in tiobarbituric acid reactive substance (TBARS) and total oxidant status (TOS) one week, and total protein concentration twenty four weeks after the attachment of orthodontic appliances was found in the saliva. The markers of antioxidant status: superoxide dismutase (SOD), catalase (CAT), uric acid (UA), peroxidase (Px), and total antioxidant status (TAS) were not changed in all periods in UWS. In SWS a significant decrease in SOD1 and CAT was found whereas Px was increased one week after treatment and UA twenty four weeks following treatment. TAS was decreased in UWS and SWS twenty four weeks after orthodontic treatment.

Oxidative status index (OSI) was elevated both in UWS and SWS one week after orthodontic treatment in comparison to the results obtained before and twenty four weeks. One week after treatment an increased concentration of nickel was also observed.
Conclusions: Orthodontic treatment modifies the oxidative–antioxidative balance in the saliva of clinically healthy subjects. Increased nickel concentration in saliva, released from orthodontic appliances, seems to be responsible for changes in the oxidative status of the saliva.

1. Introduction

The oral cavity is a very complex milieu characterised by numerous interactions between different tissues, secretions from various glands, surfaces, foods, air and microorganisms. Saliva is in the centre of the oral cavity and, to a certain degree, salivary markers reflect the condition of the oral cavity. The use of orthodontic appliances in the treatment of various maxillary dental anomalies creates a very complex environment in the oral cavity. An inflammatory response localised around the tooth, or teeth subjected to displacement is frequently observed. A large number of inflammatory mediators are involved in the response to the mechanical forces occurring during orthodontic treatment [1]. One of the biological responses to orthodontic treatment and the ensuing inflammation in the oral cavity is oxidative stress associated with an enhanced expression of proinflammatory factors [2,3].

Oxidative stress is defined as an imbalance between the production of free radicals and the body's ability to counteract or minimise their harmful effects through their neutralisation by antioxidants [4]. Reactive oxygen species (ROS), which cause oxidative damage, include both oxygen-free radicals and non-radical oxygen derivatives involved in oxygen radical production. The main ROS are hydrogen peroxide (H_2O_2), superoxide anion ($^{\bullet}O_2^-$) and hydroxyl ($^{\bullet}OH$) radicals. The resulting ROS are considered to be one of the most important factors in oxidative damage to cells and tissues by affecting the peroxidation of double-chain fatty acids, proteins and DNA as well as by increasing oxidative stress [5–7]. Oxidative stress is a causative factor in a

* Corresponding author at: Department of Orthodontics, Medical University of Bialystok, Jana Kilinskiego 1, Bialystok, Poland.
E-mail address: pbuczko@op.pl (P. Buczko).

http://dx.doi.org/10.1016/j.advms.2016.11.004
1896-1126/

number of pathophysiological conditions including gene mutations and cancer, neurodegenerative diseases, atherosclerosis, inflammatory diseases, chronic renal failure, diabetes [8–12] and some oral cavity diseases, e.g. periodontitis and squamous cell carcinoma [13–15]. Thus, evaluation of oxidative status in the oral cavity has been suggested as an important tool in the diagnosis and assessment of progression of these diseases.

In the last decade, a limited number of papers indicating that orthodontic treatment can be a factor inducing oxidative stress in saliva have been published [16–18]. The reported results are not consistent. In vitro studies by Buljan et al. [17] have demonstrated that all types of orthodontic brackets, regardless of the constituent materials, are a source of oxidative stress in murine fibroblast cells L929. A higher concentration of oxidative stress markers was observed in subjects with traditional, metal and self-ligating brackets compared to the negative control. Conventional ceramic brackets showed high viability and caused the largest increase in the number of oral mucosa cells but the weakest oxidative stress symptoms [17].

In 2009 Olteanu et al. [16] were the first researchers to study selected oxidative biomarkers in very young patients treated orthodontically. They found a statistically significant increase in the concentrations of ceruloplasmin and malondialdehyde, which reached maximum levels 24 h after treatment and 1 h after treatment for hydrogen donors, while 7 days after device attachment concentrations of the salivary markers of oxidative stress were close to the initial values. In 2014 Ozcan et al. [18] were the first researchers who evaluated the level of selected oxidative stress markers by using saliva and gingival cervicular fluid (GCF) for determining oxidative damage that man occur during orthodontic treatment as a result of aseptic inflammation. The authors found that orthodontic treatment did not change levels of the studied oxidative stress markers in saliva and GCF above the physiological limits one and six months after orthodontic appliance insertion.

It has been postulated recently that single markers can validate disease presence or prognosis, but utilising a panel of biomarkers would be more helpful and yet, estimating total oxidant status (TOS) and total antioxidant capacity (TAC) would be more appropriate [19,20]. Since an oxidant–antioxidant imbalance is the underlying principle of oxidative stress, it has been suggested that the TOS to TAC ratio should be calculated as a more accurate indicator of oxidative stress in the body [21,22]. Taking the above into account, we measured total oxidative status and an antioxidant response in the saliva of clinically healthy subjects one week and twenty four weeks after orthodontic treatment.

2. Material and methods

The study reported 60 students, but due to the fact that 15 of them were smokers, 3 had diabetes type 1 and 5 during the last 6 months has gone through a bacterial or viral infection, which was the reason for taking medication to further study 37 participants were enrolled.

Unstimulated (UWS), and stimulated whole saliva (SWS) were collected from 37 participants – dentistry students of the Medical University in Bialystok (28 female, 9 male), 21.2–24.5 years old (median – 22.3; interquartile range – 21.3–23.6). All participants were clinically stable non-smokers, with no known health problems. The subjects had not taken medication which could influence saliva composition 6 months prior to the commencement of the study and were not suffering from any systemic diseases and chronic or acute oral infections (mucositis or candidiasis). The participants were instructed and screened once a week in all aspects of oral hygiene by a qualified dentist in the month preceding the attachment of the fixed appliances. UWS and SWS were collected 30 min before bracket insertion. Conventional 3 M Victory Series steel brackets, which contain approximately 30% nickel, 15% chromium, 3% cuprum and less than 1% of other elements, were used. During the whole study period only nickel–titanium arch wires were used. Following bracket insertion, the subjects received instructions concerning the need for supplementary oral hygiene measures (orthodontic toothbrushes, dental floss, mouth rinsing). All appliances were inserted by two experienced dentists. Out of the 37 participants, 5 displayed a Class II malocclusion, 4 had crossbite and all subjects had dental crowding. No tooth extractions were performed. Written informed consent was obtained from all participants following the explanation of the purpose and nature of the study. The Ethics Committee at the Medical University of Bialystok (permission number R-I-002/67/2012) approved the study.

Study participants were instructed to refrain from food for 2 h before saliva collection, which was performed between 8 and 9 a.m. Samples of UWS were taken 10 min after rinsing the mouth with distilled water (MilliQ) under the supervision of two dentists (P.B. and M.G.), by passive spitting into a container immersed in crushed ice [23]. Saliva collected during the first minute was discarded. Subsequent portions of saliva, which were accumulated at the bottom of the mouth, were actively spat out into a plastic container every 60 s. Citric acid-stimulated whole saliva (SWS) was collected in the same manner for 5 min, following UWS collection. Stimulation with citric acid was performed by two dentists (P.B and M.G) by placing 100 μL of 1% citric acid on the posterior part of the tongue every 30 s. After measuring the volumes, saliva samples (3 mL) were centrifuged at 3000 × *g* for 20 min at 4 °C to remove cells and debris. The resulting, not stained, supernatants were divided, frozen, and kept at −80 °C until analysed [24].

Clinical examinations of the participants were performed by experienced dentists (P.B., M.G.) under standardised conditions in the Orthodontic Department at the Medical University in Bialystok, in a dental chair, using portable equipment with a fibre optic light, a suction device, and compressed air. All examinations were conducted using dental diagnostic instruments (a dental mirror, a probe, and a periodontal probe). The dental status of each subject was determined using the Decayed, Missing, Filled index (DMFT) in accordance with the WHO criteria [25]. The gingival status was assessed using the gingival index GI, and the periodontal status was established on the basis of the probing pocket depth (PPD) measurements.

Clinical examinations and saliva collection were performed by the same dentist on three occasions – immediately before the insertion of the appliances a, one week after treatment, and twenty four weeks after the attachment Cof fixed appliances. Non-stimulated saliva samples were collected first, followed by stimulated saliva sample collection. A dental examination constituted the final part of the procedure. In the case of all 37 participants, the inter-rater reliability between the principal examiner (P.B.) and another experienced dentist (M.G.) was assessed at each examination. At the first examination, the inter-rater reliability for DMFT was $r = 0.97$, for gingival index (GI): $r = 0.96$ and for Oral Hygiene Index (OHI): $r = 0.96$. At the second examination, the inter-rater reliability for DMFT was $r = 0.99$, for GI: $r = 0.99$ and for OHI: $r = 0.98$. At the third examination, the inter-rater reliability for DMFT was $r = 0.97$, for GI: $r = 0.97$ and for OHI: $r = 0.95$.

Although in vivo studies are exceptionally useful in explaining how orthodontic materials interact with oral tissues in their natural environment, interpretation of research results is usually difficult because of many factors which are not under experimental control. In our preliminary experiments we observed individual variations in the salivary markers of oxidative stress even in