Clinica Chimica Acta 478 (2018) 188–193

Contents lists available at ScienceDirect

Clinica Chimica Acta

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

A pilot study of the metabolomic profiles of saliva from female orthodontic patients with external apical root resorption

Jinglin Zhou[a,*], Huimin Hu[b], Renhuan Huang[b]

[a] Deptment of Oral and Maxillofacial Surgery, State Key Laboratory of Oral Disease, West China School of Stomatology, Sichuan University, PR China
[b] State Key Laboratory of Oral Disease, West China School of Stomatology, Sichuan University, PR China

ARTICLE INFO

Keywords:
Orthodontically induced external apical root resorption
Metabolomics
^{1}H NMR spectroscopy
Inflammation
Oxidative stress
Saliva

ABSTRACT

Background: Orthodontically induced external apical root resorption (OIEARR) is one of the most severe complications of orthodontic treatment, which is hard to diagnose at early stage by merely radiographic examination. This study aimed to identify salivary metabolic products using unbiased metabolic profiling in order to discover biomarkers that may indicate OIEARR.

Materials and methods: Unstimulated saliva samples were analyzed from 19 healthy orthodontic patients with EARR (n = 8) and non-EARR (n = 11). Metabolite profiling was performed using ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy.

Results: A total of 187 metabolites were found in saliva samples. With supervised partial least squares discriminant analysis and regression analysis, samples from 2 groups were well separated, attributed by a series of metabolites of interest, including butyrate, propane-1,2-diol, α-linolenic acid (Ala), α-glucose, urea, fumarate, formate, guanosine, purine, etc. Indicating the increased inflammatory responses in the periodontal tissues possibly associated with energy metabolism and oxidative stress.

Conclusions: The effective separation capacity of ^{1}H NMR based metabolomics suggested potential feasibility of clinical application in monitoring periodontal and apical condition in orthodontic patients during treatment and make early diagnosis of OIEARR. Metabolites detected in this study need further validation to identify exact biomarkers of OIEARR. Saliva biomarkers may assist in diagnosis and monitoring of this disease.

1. Introduction

Orthodontically induced external apical root resorption (OIEARR) is defined as a complex inflammatory process involving the breakdown of dentin, cementum, and periapical tissue, which is one of the most severely undesirable pathologic consequence of orthodontic tooth movement (OTM) [1,2]. Clinical manifestation and prevalence of OIEARR highly vary, revealed as 46% to 66% occurrence assessed by panoramic, CBCT or periapical radiographs in clinic [3,4,5], whereas > 90% occurrence by a histologic study [6]. The degree of resorption in OIEARR is mostly minor thus with little clinical significance. Moderate (3–5 mm) to severe (> 5 mm) root resorption has been reported to occur with a frequency of 2% to 20% [5].

Previous literature on the mechanism of OIEARR revealed a multifactorial etiology and individual predisposition. Genetic variations [7,8], tooth morphology, pretreatment periodontal condition and individual bone turnover have been reported as risk factors of OIEARR besides orthodontic force application [8,9].To achieve desired OTM with as fewer side-effects as possible, the cellular and molecular mechanisms of OIEARR and physical OTM should be further understood. In the periodontium tissues, several inflammatory mediators have been detected as biomarkers of OIEARR, containing interleukin (IL) family, tumor necrosis factor (TNF)-α, alkaline phosphatase, matrix metalloproteinase (MMP), prostaglandins (PGs), macrophage colony-stimulating factor (M-CSF) [1,10,11]. Possible signaling pathways have been suggested, such as the ATP/P2XR7/IL-1B in inflammatory modulation; and RANK/RANKL/OPG controlling osteoclast activation [10] as well as Wnt [12], Notch [13] pathways.

Considering the complex nature of OIEARR and lagging phase of radiographic detection (It usually detected after 3 months of OIEARR.) [14,15], effective and safe analytical platforms are required to investigate the mechanisms and provide early diagnosis of OIEARR.

In recent years, the analysis of gingival crevicular fluid (GCF) and saliva samples of patients receiving orthodontic forces has revealed

Abbreviations: OIEARR, orthodontically induced external apical root resorption; EARR, external apical root resorption; NMR, Nuclear Magnetic Resonance; OTM, orthodontic tooth movement; OPG, orthopantomogram; TSP, sodium trimethylsilyl-[2,2,3,3-2H4]-1-propionate; ROS, reactive oxygen species

* Corresponding author.
E-mail address: zhou_jl@scu.edu.cn (J. Zhou).

https://doi.org/10.1016/j.cca.2017.12.046
Received 26 November 2017; Received in revised form 22 December 2017; Accepted 28 December 2017
Available online 29 December 2017
0009-8981/ © 2017 Published by Elsevier B.V.

significant changes in terms of several cytokines and enzyme, including IL-B, aspartate aminotransferase, tartrate-resistant acid phosphatase, alkaline phosphatase and so on [16,17]. Compared to GCF, saliva is more easily accessible and can also provide abundant information of the periodontium with sensitive and high-throughput technique, possibly due to the open communication between GCF and saliva.

Metabolomics is top-down platform that monitors the global metabolite dynamics of a living system in respond to external or internal stimuli, with high sensitivity and specificity. Non-targeted metabolomic approaches are in wide application to discover new biomarkers and explore the mechanisms of various human diseases [18,19]. Salivary metabolomics have distinguished between health and periodontitis [20], caries [21] and inflammation [22], while no previous studies reported the global salivary metabolomics in OIEARR. High-resolution Nuclear Magnetic Resonance (NMR) spectroscopy is now a well-established metabolomic tool which is virtually non-invasive and provides simultaneous multicomponent information. NMR has been widely used in analyzing human biofluids.

Herein, we supposed that the inflammatory reaction of OIEARR may lead to a variation in the downstream metabolite profile. In the this preliminary study, the ^{1}H NMR spectroscopy was used to compare metabolic variations in saliva from orthodontic patients with clinically detectable external apical root resorption and that from healthy patients with normal tooth morphology. We aimed to assess the feasibility of applying 1HNMR in detecting OIEARR in clinic and the quality of saliva as the analytic samples. The secondary aim was to identify typical biomarkers of EARR in saliva samples, which might bring new insights into the mechanism research and early diagnosis of OIEARR.

2. Materials and methods

2.1. Subjects

We recruited participants from adult female patients who finished or were about to finish orthodontic treatment at outpatient department of West China Hospital of Stomatology, Sichuan University Department of Orthodontics, West China Hospital of Stomatology, Sichuan University. All of the participants were fully informed and consented.

Inclusion criteria were: (1) patients aged between 18 and 35 y receiving (straight-wire technique) fixed orthodontic treatment; (2) with no orthodontic retreatment, no systemic illness, non-smoker, not pregnant; no pharmacological treatment or antibiotic therapy during or up to 3 months before the study; (3) with stable and healthy frontal teeth, no root canal therapy or trauma injury before; (4)with no periodontitis, no active caries, absence of extensive dental restorations (e.g., crowns or bridges) or removable partial dentures; (5) with full medical records; (6)consent and willing to participate the study. Individuals were excluded if: (1) patients were overweight or who had a rich dinner (high-fat, high-sugar) in recent 3 days before sample collection; (2) had congenital missing teeth (except the third molar), hyperdontia, or alveolar bone defect; (3) the orthopantomogram (OPG) image was not clear enough for measurement. The protocol was approved by the Ethics Committee of West China Stomatology Hospital.

2.2. Measurement and analysis of tooth resorption

Orthodontic patients at our apartment took OPG two times, prior to (T1) and after treatment (T2) respectively. All radiographs were taken with the same device. The dental panoramic radiographs were measured in Planmeca ProMax with an accuracy of 0.01 mm. All root and crown measurements were taken twice in each radiograph and measured by one examiner. Since the incisor roots are the most frequently reported resorption site, we focused on the root apical morphology of central incisors. The following variables were measured with modification of the methods brought by Krieger [5]: a cross defined by two intersecting lines, long axis and mesial-distal line of the tooth. Before the main measurements the examiner was calibrated by measuring ten different radiographs five times in random order and a minimum of two weeks in between the measurements.

2.3. Sample collection and preparation

Unstimulated human saliva samples were obtained from a total of 19 eligible participants (8 EARR patients, 11 controls) using the sampling System "Salivette" (Sarstedt, Germany) with a low-adsorptive polypropylene-based swab. Participants were requested to refrain from eating, drinking, tooth-brushing etc. after waking in the morning on the sampling day. The sampling processes were all conducted during 8:00–10:00 a.m. The subjects sat, rinsed their mouth with tri-distilled water (30 s × 3 times), then rested for at least 5 min.

Then the swab was placed in the occlusal space of right mandibular molars without chewing for about 3 min to collect unstimulated saliva and the swab was collected in the supplied polypropylene centrifuge tube, transported to the laboratory on ice and then centrifuged immediately (2000 × g for 10 min) to obtain saliva without cells and debris. The resulting liquid supernate (1 ml for each participant) was collected and aliquoted for ^{1}H NMR analysis. Saliva samples were stored at −80 °C before analyzed.

2.4. ^{1}H NMR analysis

Prior to the measurements, saliva samples were thawed and 500 μl of each aliquot was mixed with 50 μl D_2O. Sodium trimethylsilyl-[2,2,3,3-2H4]-1-propionate (TSP) was added as an internal chemical shift standard The pH values of each salivary supernatant were determined prior to ^{1}H NMR analysis (range 7.2 to 7.6). All samples were performed on a Bruker Avance II 600 MHz spectrometer at 300 K (Bruker Biospin). Two pulse sequences were used to obtain the NMR spectrum: selective presaturation pulse sequence (Bruker Biospin, Germany) for water suppression (located in δ 4.8 ppm) and CPMGPR1D pulse sequence (Bruker Biospin, Germany) for attenuating the broad protein signal in the sample. The free induction decay (FID) was collected with 64 K data points and 128 scans. Both phase and baseline corrections of the NMR spectra were carefully checked with the TopSpin 1.3 (Bruker Biospin). Details of the parameters were described in our previous study [23].

2.5. Data analysis

All signals of the 19 samples' were located in the range of δ 0–8.5 ppm in spectral region. Each spectrum was subdivided into 0.04 ppm integral segments and integrated, leaving out the region 4.5–5.0 ppm to avoid the influence of water signal. The reduced spectra consisted of 187 independent variables which were normalized to the unit area with the appropriate weighting coefficients in Excel (Microsoft), and further analysis was performed using SIMCA-P software package (ver11.0, Umetrics AB).

Partial least squares discriminant analysis (PLS-DA) was conducted using SIMCA-P 11.0 (Umetrics AB) with the application of mean centering and unit variance scaling. Metabolite were concentrated using value of Variable Importance for the Projection (VIP, which > 1 indicating a useful one) and predictive model (which be set Y value to separate data in model base on regression coefficient analysis). Significance was set 0.05 for all tests.

3. Results

3.1. Baseline characters and incidence of EARR

The demographic information for participants is demonstrated in Table 1.

Download English Version:

https://daneshyari.com/en/article/8309750

Download Persian Version:

https://daneshyari.com/article/8309750

Daneshyari.com