Archives of Oral Biology 72 (2016) 92–98

Contents lists available at ScienceDirect

Archives of Oral Biology

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

Biomarker levels in gingival crevicular fluid of subjects with different periodontal conditions: A cross-sectional study

Qian Zhang, Bin Chen, Dinggui Zhu, Fuhua Yan*

Nanjing Stomatological Hospital, Medical School of Nanjing University, 30 Zhongyang Road, Nanjing, Jiangsu 210008, China

ARTICLE INFO

Article history:
Received 9 August 2015
Received in revised form 31 May 2016
Accepted 19 August 2016

Keywords:
Biomarkers
Gingival crevicular fluid
Periodontitis

ABSTRACT

Objective: To compare five biomarker levels in gingival crevicular fluid (GCF) in different tooth-sites of subjects with healthy periodontium, aggressive periodontitis and severe chronic periodontitis, and to evaluate the value of these biomarker levels for diagnosis of the type and activity of periodontitis.
Materials and methods: Prior to therapy, GCF samples were collected using filter paper strip at different tooth-sites of 10 subjects with healthy periodontium (H), 15 with severe chronic periodontitis (CP) and 15 with aggressive periodontitis (AgP). The strips were weighed and the periodontal clinical parameters were recorded. Levels of interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), C-reactive protein (CRP), and alkaline phosphatase (ALP) in GCF were assessed by enzyme-linked immunosorbent assay (ELISA).
Results: The volumes of the GCF samples obtained in CP and AgP subjects were significantly higher than those from subjects with healthy periodontium ($P < 0.05$). Levels of IL-6, TNF-α, CRP and ALP were significantly higher in the untreated disease sites in the CP and AgP groups compared to those in control sites in the H group, while IL-10 levels were lower in the CP and AgP groups than those in the control sites in the H group. However, the levels of all five biomarker levels showed significant correlation with the clinical parameters.
Conclusion: The measurement of five biomarker levels in GCF may facilitate overall screening of periodontitis patients in epidemiological studies and allow estimation of periodontitis activity.

1. Introduction

Periodontitis is a common inflammatory disease, which causes connective tissue and bone damage that can result in the loss of the tooth. Epidemiological studies have shown that the majority of the adult population has periodontitis of varying degrees of severity (Burt, 2005). Periodontitis is the primary reason for tooth loss in adults caused not only by the breakdown of the tooth supporting tissues, and is also a potential risk factor for some systemic diseases (Mealey, 2000). Recent studies have confirmed the existence of an interaction effect between periodontitis and diabetes, and periodontitis is also a potential risk factor for atherosclerosis and cardiovascular disease (Tüter, Kurtis, & Serdar, 2007). Furthermore, adverse outcomes of pregnancy and rheumatoid arthritis are also closely related to periodontal diseases (Mealey, 1999). Due to the prevalence of periodontitis, strategies for early diagnosis and treatment are now a focus of research (Buduneli & Kinane, 2011).

Traditionally, the diagnosis and evaluation of periodontitis are based on periodontal parameters, such as the probing pocket depth (PD), bleeding on probing (BOP), plaque index (PI), gingival recession (GR), clinical attachment loss (CAL) and radiographic findings (Khongkhunthian et al., 2014). However, these parameters do not reflect the early periodontal diseases or provide a basis for evaluation of assess periodontitis activity (Oh, Hirano, Takai, & Ogata, 2015). Therefore, new methods are required to detect the active disease sites and to monitor the disease progression.

Gingival crevicular fluid (GCF) is a transudate derived from serum that is found in the gingival sulcus (AlRowis et al., 2014). Most of the constituents of serum are present in GCF, and more than 65 components represent potential biomarkers that can be used to assess periodontal disease initiation and progression (Gupta, 2012; Loos & Tjoa, 2005). Periodontitis depends on the complicated interactions between pathogenic bacteria and the host immune responses. The constituents of GCF change due to the inflammatory response induced in periodontal disease and some of cytokines are selectively upregulated. The destruction of periodontal tissues occurs due to excessive production of proinflammatory cytokines or the imbalance between the

* Corresponding author.
E-mail addresses: yanfh@nju.edu.cn, fhyan2005@126.com (F. Yan).

http://dx.doi.org/10.1016/j.archoralbio.2016.08.020
0003-9969/

proinflammatory and the anti-inflammatory cytokines (Cetinkaya, Guzeldemir, Ogus, & Bulut, 2013). To date, many cytokines have been proposed as diagnostic or predictive biomarkers of periodontal disease pregression and therapeutic outcome (Fiorini et al., 2013; Goutoudi, Diza, & Arvanitidou, 2004; Recker et al., 2015; Reis et al., 2014; Thunell et al., 2010). However, the role of biomarkers such as cytokines, proteins and enzymes in GCF has not been investigated in a single study. Therefore, in the present study, we evaluated five biomarkers [proinflammatory interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), anti-inflammatory interleukin-10 (IL-10), as well as alkaline phosphatase (ALP) and C-reactive protein (CRP)] in GCF in subjects with three different periodontal conditions.

The aim of the present study was to determine the levels of IL-6, IL-10, TNF-α, CRP and ALP in the GCF of periodontally healthy individuals as well as in patients with aggressive and severe chronic periodontitis. We also investigated the relationship between these biomarkers and periodontal conditions, to evaluate the relationship between levels of these biomarkers and disease severity to determine the potential of these markers as diagnostic and prognostic indicators of periodontitis.

2. Materials and methods

2.1. Subject recruitment

A total of 40 participants were recruited from the Department of Periodontology, Nanjing Stomatological Hospital, Medical School of Nanjing University, China between 2013 and 2014. Participants were assigned to the healthy periodontium group (H) (n = 10), the severe chronic periodontitis group (CP) (n = 15) and the generalized aggressive periodontitis group (AgP) (n = 15). This study was approved by the Human Ethic Committee of Nanjing Stomatological Hospital, Medical School of Nanjing University (Ethic Committee no.: 2013NL-007) and registered with the Chinese Clinical Trial Registry (Registration no.: ChiCTR-OCH-13004679). The objective and the content of the study were explained by our specialists to all participants before enrolment, and the informed consent was signed by each individual.

Exclusion criteria were as follows: patients with systemic diseases (diabetes mellitus, malignancy, cardiovascular and cerebrovascular diseases), patients who received any periodontal treatment during the previous six months or used antibiotics in the previous three months, patients who had a history of orthodontic treatment, patients with a history of smoking and pregnant women.

The selection and the diagnosis of the patients was based on the clinical and radiographic criteria proposed by the American Academy of Periodontology for the Classification of Periodontal Disease (Armitage, 1999).

Group H comprised four males and six females, aged 22–35 years, with probing pocket depth (PD) <3 mm, no clinical attachment loss (CAL), no evidence of bone loss radiographically and $\leq$20% of the sites with bleeding on probing (BOP).

The CP group included four males and eleven females, aged 38–52 years. Patients included in this group had visible gingival inflammation, radiographic evidence of bone loss, CAL ≥ 5 mm and PD ≥ 6 mm in $\geq$30% of intraoral sites. The severity of periodontal damage was in accordance with the local factors (dental plaque and calculus).

The AgP group consisted of seven males and eight females, aged 23–33 years. These patients showed severe periodontal destruction and loss of attachment ≥ 5 mm and PD ≥ 6 mm (at least three of these diseased teeth were not first molars or incisors). Unlike the CP group, the severity of periodontal damage was not consistent with the local factors.

2.2. Clinical periodontal examination

All clinical examinations were performed by one experienced examiner and participants were assigned to the three groups based on their periodontal conditions.

Each participant received a comprehensive periodontal examination, which comprised of probing pocket depth (PD), bleeding on probing (BOP), plaque index (PI) (Loe, 1967), gingival recession (GR), clinical attachment loss (CAL), and tooth movement (TM). Every tooth was measured at six sites: mesiobuccal, buccal, distobuccal, mesiolingual, lingual and distolingual. PD and GR were measured using a periodontal probe (Hu-Friedy Co, USA), the two measurements were combined and recorded as CAL, which represents the distance between the bottom of the pocket and the cemento-enamel junction. BOP during the examination was recorded as present or absent. Information about the participant's brushing and flossing frequency, past history of periodontal therapy and family history of periodontal disease were also recorded.

2.3. Collection of GCF

GCF samples were collected two days after the periodontal examination to minimize the effects of the examination. In the H group, four sites per person were chosen randomly, while in the CP and AgP groups, two mild periodontal disease sites (CAL: 1–2 mm) and two severe periodontal disease sites (CAL ≥ 5 mm) were selected. The selected tooth-sites were isolated with sterile cotton rolls, and the clinically visible supragingival plaque was removed using a curette, take care to avoid touching the marginal gingiva. Sites were gently dried with an air syringe before a sterile paper strip (size: 2×8 mm, Whatman, USA) was inserted into the gingival crevice until slight resistance was felt, and held in position for 30 s. After collection of the GCF, the strip was immediately placed into a pre-weighed sterile Eppendorf tube. Two minutes later, the procedure was repeated in the same site to obtain a second GCF sample, which was placed in the same tube as the first sample. Strips contaminated with blood or saliva were discarded. After GCF collection, the tube containing two strips was re-weighed with a precision electronic balance (Mettler-Toledo, XS205, China), and the difference between the two values was recorded as the weight of the GCF. The calculation of GCF volume was made considering the specific gravity of GCF was almost 1 g/ml. The tubes were stored at $-80\,^{\circ}$C for subsequent analysis.

2.4. Assessment of IL-6, IL-10, TNF-α, CRP and ALP

During the analysis, each Eppendorf tube was placed at room temperature for one hour prior to the addition of 300 μl phosphate buffered solution (PBS). Following centrifugation, the supernatant was removed for determination of IL-6, IL-10, TNF-α, CRP and ALP levels in the GCF by enzyme-linked immunosorbent assay (ELISA) using commercially available kits according to the manufacturers' protocols(IL-6, IL-10, TNF-α, CRP kits, R&D Systems, USA; ALP kit, Cloud-Clone, USA). Biomarker levels were considered to be undetectable in some sites if the levels were below the detection limits of the assay.

2.5. Statistical analysis

Data were analyzed with Statistical Software of SPSS 20.0 (Chinese version). Graphs were generated in Graph-Pad Prism version 5 for Windows. The differences in clinical parameters between groups were analyzed by one-way ANOVA. Descriptive statistics were computed for all biomarker outcomes. Some outliers and extremes were excluded from our analysis. Since