Archives of Oral Biology 60 (2015) 1503–1509

Contents lists available at ScienceDirect

Archives of Oral Biology

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

Novel biomarkers of periodontitis and/or obesity in saliva—An exploratory analysis

Erica N. Recker[a], Kim A. Brogden[a,b], Gustavo Avila-Ortiz[b], Carol L. Fischer[a], Keyla Pagan-Rivera[c,d], Deborah V. Dawson[a,c], Katherine M. Smith[b], Satheesh Elangovan[b,*]

[a] Dows Institute of Dental Research, University of Iowa College of Dentistry, Iowa City, IA 52242, USA
[b] Department of Periodontics, University of Iowa College of Dentistry, Iowa City, IA 52242, USA
[c] Biostatistics and Research Design, University of Iowa College of Dentistry, Iowa City, IA 52242, USA
[d] Department of Biostatistics, University of Iowa College of Public Health, Iowa City, IA 52242, USA

ARTICLE INFO

Article history:
Received 3 March 2015
Received in revised form 3 June 2015
Accepted 7 July 2015

Keywords:
Saliva
Diagnostics
Periodontal disease
Bbesity

ABSTRACT

Objective: Recent studies point to the clinical and research utility of saliva as a valuable diagnostic aid for monitoring periodontal health. The objectives of this study were to detect novel biomarkers attributed to chronic inflammation in saliva and to determine if the levels of these markers correlate with severity of periodontitis and with standard obesity measures in participants in a periodontal maintenance program.
Design: In this cross-sectional assessment of 63 participants, unstimulated whole saliva was collected after recording anthropometric and clinical parameters of obesity and periodontitis, respectively. The levels of interleukin-1 receptor antagonist (IL-1ra), sCD40L, granzyme B and alpha-fetoprotein (AFP) in saliva were determined using multiplex proteomic immunoassays. The correlation between the four tested biomarker concentrations and obesity/periodontal measures was determined.
Results: Positive correlation between fat% and granzyme B levels ($r = 0.292$; $p = 0.020$) and negative correlation between BMI and sCD40L ($r = 0.256$; $p = 0.043$) was observed. In addition, positive correlation between severity of periodontal disease and levels of IL1-ra ($r = 0.253$; $p = 0.046$) and negative correlation between periodontitis severity and sCD40L salivary levels ($r = 0.272$; $p = 0.031$) was noted. None of the above correlations remained statistically significant after multiple comparisons adjustment. After adjustment for clinical covariates, the relationship between sCD40L and periodontal severity remained suggestive ($p = 0.081$).
Conclusions: Levels of four novel biomarkers of periodontitis were detectable in saliva of subjects enrolled in a periodontal maintenance program. Prospective studies with larger sample sizes and other populations are warranted to explore the diagnostic applicability of these markers.

1. Introduction

Obesity and chronic periodontitis are two highly prevalent conditions of inflammatory nature. The number of people in developed nations who are obese is at rise. In the United States, it was estimated that, in 2009–2010, 35.5% of adult men and 35.8% of adult women were obese (Flegal, Carroll, Kit, & Ogden, 2012). Periodontal disease is also highly prevalent, affecting approximately 47% of the United States population over the age of 30, according to 2009–2010 NHANES data (Eke, Dye, Wei, Thornton-Evans, & Genco, 2012). A recent update by the same group examined data from 2011 to 2012, including 7066 adults over the age of 30 (Eke et al., 2015). This update showed findings statistically similar to the data published in 2012. This study stated that 44.7% of adults in the United States had periodontitis. For the combined period of 2009–2012, 45.9% of adults over the age of 30 in the United States have periodontitis, with 8.9% having disease classified as severe (Eke et al., 2015). There is growing evidence that obesity may play an important role in the pathogenesis of periodontal disease. Recently published longitudinal studies and systematic reviews/meta-analyses clearly underscored this association (Chaffee & Weston, 2010; Gorman et al., 2012). In addition, our group has reported a positive correlation between select obesity measures and the expression of

* Corresponding author at: Department of Periodontics The University of Iowa College of Dentistry, S464, Iowa City, IA 52242, USA.Fax: +1 319 335 7239.
E-mail address: satheesh-elangovan@uiowa.edu (S. Elangovan).

http://dx.doi.org/10.1016/j.archoralbio.2015.07.006
0003-9969/

pro-inflammatory mediators in peri- implant sulcular fluid (Elangovan et al., 2014).

Predicting the onset and/or progression of chronic periodontitis solely based on clinical and radiographic assessments has inherent limitations, given the site-specific and non-linear nature of this local inflammatory condition. Furthermore, the influence of concomitant systemic inflammatory conditions, such as obesity, on the pathogenesis of chronic periodontitis cannot be fully understood on the basis of conventional diagnostic methods. The analysis of biologic fluids to identify markers that may enable clinicians to accurately predict the onset and progression of chronic periodontitis has been proposed as a complementary diagnostic method to overcome some of the aforementioned limitations (Giannobile, 2012). These advanced diagnostic approaches have the potential to allow clinicians to design a more effective, personalized treatment plan to either prevent or treat distinct forms of chronic periodontitis that have been traditionally considered to be the same, given its phenotypical similarities (Garcia, Kuska, & Somerman, 2013). Salivary diagnostics, one of these emerging diagnostic tools, has been well received because of the ease of collection, established laboratory protocols for analysis and the availability of extensive data from past studies using saliva that can be used for comparisons (Pfaffe, Cooper-White, Beyerlein, Kostner, & Punyadeera, 2011; Schulz, Cooper-White, & Punyadeera, 2013).

The diagnostic or prognostic value of multiple salivary markers has been studied for chronic periodontitis (Kinney et al., 2011). In a separate case-control study, 98 inflammatory biomarkers were assessed in gingival crevicular fluid (GCF), of which only four novel biomarkers were significantly elevated in the chronic periodontitis group (Starr et al., 2014). These four biomarkers are interleukin-1 receptor antagonist (IL-1ra), sCD40L, granzyme B and alpha-fetoprotein (AFP). All of them were implicated to play a pivotal role in chronic inflammatory conditions(Baena-Fustegueras et al., 2013; El Mesallamy et al., 2014; Potapovich et al., 2009). For example, high serum levels of IL-1ra, which inhibits the pro-inflammatory effect of IL-1, were found in patients with metabolic syndrome (Salmenniemi et al., 2004). Specific biological functions of the selected biomarkers are listed in Table 1. The primary objective of this cross-sectional assessment was to detect the baseline levels of these four novel biomarkers in saliva obtained from periodontal maintenance patients and to correlate these values with severity of periodontitis. Secondarily, the correlation between the levels of these novel markers and body fat indices was also assessed.

2. Materials and methods

2.1. Participant identification and recruitment

This project stems from a parent study that evaluated the correlation between obesity measures and levels of inflammatory biomarkers in sulcular fluid obtained around dental implants (Chaffee & Weston, 2010). The parent study was conducted after obtaining Institutional Review Board's approval from the University of Iowa's Human Subjects Office (IRB # 201109878). Briefly, participants enrolled in the College of Dentistry periodontal recall program and having at least one rough surface implant in function for a minimum of 6 months were identified by searching the electronic health record (EHR). Data and sample collection were done in eligible participants between June 2012 and April 2013. To be eligible for the parent study, participants had to be 18 years of age or older, current non-smokers and had to be enrolled in a collegiate periodontal maintenance program. Participants with aggressive periodontitis and pregnant or nursing women were not eligible. Also, patients who were completely edentulous, had blade-type or smooth surface implants, or who had taken medications such as antibiotics and anti-inflammatory agents for 3 months prior to the study visit were excluded. Participant's medical history was thoroughly reviewed during the initial telephone calls that we made to assess eligibility and also during the study visit.

2.2. Body composition and systemic evaluation

Before clinical examination and sample collection, height (in meters), weight (in kilograms) and waist circumference (in centimeters) were measured and recorded, as described elsewhere (Chaffee & Weston, 2010). Briefly, body mass index (BMI) was calculated using the Quetelet Index [weight (kilograms)/height (meter) (Eke, Dye et al., 2012)]. Waist circumference (WC) of the subject was then recorded using measuring tape (RJL System, Clinton Township, MI, USA). Body fat content (%) was measured non-invasively using a bioimpedance-based body fat analyzer (RJL System, Clinton Township, USA). At the same visit, blood pressure and fasting blood glucose level (One Touch Ultra 2 Blood Glucose Meter, Milpitas, CA, USA) were also measured.

2.3. Saliva collection and analysis

Unstimulated whole saliva samples were collected from patients who fasted for at least one hour. Participants were asked to passively drool onto a 50 mL centrifuge tube (placed on ice). The participants were given 15 min to get a total volume of 2 mL. If the 2 mL volume was achieved before 15 min, the collection was stopped. The samples were aliquoted and stored in −80 °C freezer for later use. At the end of the clinical phase, all saliva samples were removed from the −80 °C freezer and thawed on ice. Particulates and debris in each sample were pelleted by centrifugation at 16,100 RCF (13,200 RPM, Eppendorf, 5415D centrifuge, Brinkmann Instruments, Inc., Westbury, NY) for 5 min at 24 °C. The supernatants were removed and held on ice. The pellets were discarded.

Table 1
Biomarkers assessed in this study and their biological functions.

Biomarkers	Functional relevance (Refs.: Schulz et al., 2013; Kinney et al., 2011; Starr et al., 2014; Baena-Fustegueras et al., 2013)
Alpha-fetoprotein	• Modulate inflammatory events in human skin • Used to detect developmental abnormalities and cancers
Granzyme B	• Induces inflammation by stimulating cytokine release • Higher serum levels in obese patients
IL-1 receptor antagonist	• Inhibits pro-inflammatory effect of IL-1α/IL-1β • Higher levels in metabolic syndrome (obesity is a component)
sCD40L	• Regulates immune/inflammatory functions • Implicated in the pathophysiology of severe chronic inflammatory diseases • Higher serum levels in obese patients