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The effect of initial periodontal treatment on plasma, gingival crevicular fluid and salivary levels of 8-hydroxy-deoxyguanosine in obesity

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ABSTRACT

Objective: Recent studies have shown adverse effects on the periodontium from the increased production of reactive oxygen species (ROS) in obesity. The purpose of this study was to investigate the effects of obesity on 8-hydroxy-deoxyguanosine (8-OHdG) levels in the bodily fluids of patients with and without periodontal disease and to evaluate changes after initial periodontal treatment.
Design: Forty-five obese individuals and 45 normal-weight individuals were included in this study. Obese and normal-weight groups were classified into three sub-groups: chronic periodontitis (CP), gingivitis (G) and periodontally healthy controls (CTRL). Gingival crevicular fluid (GCF), plasma, saliva samples and clinical measurements were obtained at baseline and a month after initial periodontal treatment. Levels of 8-OHdG were analysed by ELISA.
Results: While plasma 8-OHdG levels were significantly higher at baseline in the obese patients with periodontal disease than in the normal-weight individuals ($P < 0.05$), no significant differences in GCF and saliva 8-OHdG levels were found ($P > 0.05$). GCF and salivary 8-OHdG levels in obese patients with G and CP were significantly higher than in CTRL groups at baseline ($P < 0.05$). After treatment, 8-OHdG levels were decreased in all groups with periodontal disease ($P < 0.01$). Statistically significant positive correlations were observed between GCF 8-OHdG levels and GI in all the groups ($P < 0.001$).
Conclusions: The significant increase of plasma 8-OHdG levels in obese patients did not correlate with saliva and GCF 8-OHdG levels when compared to normal-weight individuals. Periodontal treatment had a positive effect on the periodontal parameters and 8-OHdG levels of both obese and normal-weight individuals.

1. Introduction

Obesity is a medical condition characterised by abnormal or excessive deposits of fat in the adipose tissue, which may have an adverse effect on health (Bullon, Newman, & Battino, 2014). Several studies have reported a significant positive relationship between obesity and periodontal disease and suggested that obesity is a major risk factor for periodontal disease (Ritchie & Kinane, 2003; Boesing, Patino, Da Silva, & Moreira, 2009; Chaffee & Weston, 2010; Shimazaki et al., 2010; Zuza et al., 2011; Dahiya, Kamal, & Gupta, 2012). The underlying biological mechanisms of the way in which obesity affects the periodontium are currently poorly understood. However, it is known that adipocytes act as an active organ by secreting various proinflammatory cytokines and hormones, contributing to the pathogenesis of periodontal diseases (Jagannathachary & Kamaraj, 2010; Zimmermann, Bastos, Gonçalves, Chambrone, & Duarte, 2013). Adipose tissue secretes several bioactive substances, such as reactive oxygen species (ROS) (Matsuzawa-Nagata et al., 2008; Suresh & Mahendra, 2014). It has been stated that obesity leads to excessive ROS in adipose tissue and, as a result, increases the amount of circulating ROS (Mohora et al., 2006). Recent studies have shown that increased oxidative stress and decreased anti-oxidative status play critical roles in the progression of periodontal disease (Chapple & Matthews, 2007; Tamaki et al., 2009). Obesity is a disease process that is defined as 'increased chronic oxidative stress status'

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(Higdon & Frei, 2003). Tomofuji et al. (2009) emphasised that obesity may affect periodontal health by inducing oxidative damage to the gingiva through the increased production of circulating ROS.

The most common stable product of oxidative DNA damage caused by ROS is 8-hydroxy-deoxyguanosine (8-OHdG) (Chapple & Matthews, 2007). Several studies have demonstrated that 8-OHdG in bodily fluids can act as a biomarker of oxidative DNA damage in periodontal diseases as well as a measure for evaluating the effect of periodontal treatment (Sezer, Ciçek, & Canakçi, 2012; Chandra et al., 2013). Also, the levels of 8-OHdG showed that body mass index (BMI) can affect DNA damage, which is associated with oxidative stress in overweight individuals (Hofer, Karlsson, & Möller, 2006).

Tomofuji et al. (2009) showed that gingival 8-OHdG levels were higher in obese rats than in lean rats. Similar results were reported in obese rats that were fed a high-fat diet and received no exercise (Azuma et al., 2011). Results of animal studies have indicated that the increase of ROS in obesity causes acute inflammation in the periodontal tissue, though this inflammation is not a direct equivalent of the chronic disease in humans (Tomofuji et al., 2009; Azuma et al., 2011). However, no studies have investigated the effect of obesity-induced oxidative stress in patients with periodontal disease.

In the present study, we hypothesised that high circulating ROS levels may increase oxidative stress levels in the GCF and saliva in obese patients with periodontal disease, and thus, that periodontal therapy could have positive effects on ROS levels. The aim of this study was to evaluate clinical periodontal parameters and to analyse 8-OHdG levels in saliva, GCF and plasma in obese and normal-weight patients with periodontal disease at baseline and after initial periodontal treatment.

2. Materials and methods

2.1. Study population

The protocol for the study was approved by the Ethics Committee of the Faculty of Medicine (2013-25-12/02), Bulent Ecevit University, Turkey, in accordance with the Helsinki Declaration of 1975, as revised in 2002. Individuals were informed about the protocol of the study and gave their written consent for the described procedures. Individuals were selected from a population of people who had received periodontal treatment at the Periodontology Department of the Faculty of Dentistry, Bulent Ecevit University, from March 2013 to January 2014. Selected patients were directed to the Endocrinology Department of the Faculty of Medicine, Bulent Ecevit University. Obesity was diagnosed by using body mass index (BMI) (Dahiya et al., 2012). BMI was categorised using the World Health Organization (WHO) classification: normal weight was identified as BMI = 18.50–24.99 kg/m^2; obese $\geq$ 30 kg/m^2 (Linden, Patterson, Evans, & Kee, 2007)

Ninety individuals (43 males and 47 females, aged 25–60 years; mean age: 0.30 $\pm$ 8.66 years) fulfilled the inclusion criteria (45 obese and 45 normal-weight). Both the obese and the normal-weight groups were classified into three sub-groups: (1) periodontal healthy, (2) gingivitis and (3) chronic periodontitis. The diagnoses were based on their periodontal conditions as outlined in the criteria proposed by the 1999 International World Workshop for a Classification of Periodontal Disease and Conditions (Armitage, 1999). The selected individuals had a minimum of 20 natural teeth, excluding third molars. The study included 15 individuals with obese-chronic periodontitis (O-CP; 8 males and 7 females, aged 34–60 years; mean age: 47.13 $\pm$ 7.17 years), 15 individuals with normal-weight CP (CP; 8 males and 7 females, aged 26–51 years; mean age: 38.47 $\pm$ 7.50 years), 15 individuals with obese-gingivitis (O-G; 7 males and 8 females, aged 25–55 years; mean age: 35.73 $\pm$ 7.23 years), 15 individuals with normal-weight gingivitis (G; 8 males and 7 females, aged 25–53 years; mean age: 31.53 $\pm$ 6.80 years), 15 individuals with obese-periodontally healthy controls (O-CTRL; 7 males and 8 females, aged 25–50 years; mean age: 41.33 $\pm$ 6.47 years) and 15 individuals with normal-weight periodontally healthy controls (CTRL; 8 males and 7 females, aged 25 to 33 years; mean age: 29.60 $\pm$ 2.30 years).

2.2. Clinical measurements and intra-examiner reproducibility

The periodontal status of patients was determined by measuring the probing depth (PD), clinical attachment level (CAL), gingival index (GI) (Löe & Silness, 1963), bleeding on probing (BOP) (Ainamo & Bay, 1975), and plaque index (PI) (Silness & Löe, 1964). The level of periodontal bone loss was determined by taking full-mouth periapical radiographs. All the clinical parameters were measured on six sites per tooth (mesiobuccal, distobuccal, midbuccal, mesiolingual, distolingual, and midlingual) using a William's periodontal probe (Hu-Friedy, Chicago, IL, USA) calibrated in millimetres by the same examiner (FÖD). Prior to the actual measurement, 10 individuals were randomly selected and used to calibrate the investigator. The investigator evaluated the individuals on two separate occasions, 48 h apart. Calibration was accepted, if measurements at baseline and at 48 h did not differ more than 10% at the millimetre level (Schwarz, Bieling, Latz, Nuesry, & Becker, 2006).

2.3. Inclusion criteria

Inclusion criteria for the selection of patients were: (1) non-smokers who had never smoked; (2) no history of systemic disease; (3) had not undergone periodontal treatment or taken medicine for at least 6 months before the study; (4) no pregnancy or lactation; (5) no alcohol or antioxidant vitamin consumption; (6) GI = 0, PD and CAL $\leq$ 3 mm, with no signs of attachment and bone loss as demonstrated by clinical and radiographic examination for the periodontally healthy groups; (7) the presence of BOP in at least 50% of the total gingiva, no clinical signs of periodontitis, and no radiographic evidence of alveolar bone loss for the gingivitis groups; and (8) clinical signs of inflammation (red colour and swelling of the gingival margin) GI $\geq$ 2, PD and CAL $\geq$ 5 mm, and bone loss affecting >30% of the existing teeth on clinical and radiographic examination for the chronic periodontitis groups.

2.4. Collection of samples

All the samples were obtained in the morning following an overnight fast, during which patients were requested not to eat or drink anything except water. Before sample collection, the individuals were checked for their adherence to protocol.

Whole saliva samples were collected prior to gingival crevicular fluid samples and before clinical periodontal measurements were taken. Patients' mouths were rinsed with distilled water, and unstimulated salivary samples were collected by patients expectorating into disposable tubes (Navazesh, 1993). About 2 ml of whole saliva were immediately centrifuged to remove cell debris (10,000 $\times$ *g* for 10 min). The supernatants (50 μl each) were stored at $-40\,^{\circ}C$ until they were analysed.

In order to avoid irritation, GCF samples were collected two days after the clinical measurements were taken, in the morning between 8:00 and 10:00. GCF samples were collected from a mesiobuccal and disto-palatal site on each tooth (molars, premolars, canines/incisors). In the CP group, the samples were