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Do salivary and serum collagenases have a role in an association between obstructive sleep apnea syndrome and periodontal disease? A preliminary case–control study

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ABSTRACT

Objectives: Despite increasing evidence for an association of obstructive sleep apnea syndrome (OSAS) and periodontal disease, the pathophysiological linking mechanisms remain unclear. This study aims to evaluate the salivary and serum matrix metalloproteinase-2, -8, -9 (MMP-2, -8, -9), tissue inhibitor of matrix metalloproteinase-1 (TIMP-1), myeloperoxidase (MPO), neutrophil elastase (NE), neutrophil gelatinase-associated lipocalin (NGAL), as well as degree of activation of MMP-2, -9 of patients with and without OSAS.

Design: A total of 50 individuals were included in the study. There were 13, 17 and 20 individuals, respectively in the control (non-OSAS) group, mild-to-moderate OSAS and severe OSAS groups. Saliva, serum samples and clinical periodontal parameters were collected. Biofluid samples were analysed by immunofluorometric assay (IFMA), enzyme-linked immunosorbent assay (ELISA), western immunoblotting and gelatine zymography. Statistical analyses were performed using D'Agostino–Pearson omnibus normality test, Kruskal–Wallis test and Spearman rho rank correlation analysis.

Results: There were no statistically significant differences in clinical periodontal parameters between the study groups. Salivary NE and proMMP-2 levels were significantly lower in the OSAS groups than the control group ($p < 0.05$). Serum proMMP-9 concentration and the degree of MMP-9 activation in saliva were significantly lower in the severe OSAS group than the control group ($p < 0.05$). There were significant correlations between salivary and serum proMMP-9 and -2 concentrations ($p < 0.05$). Serum proMMP-2, NE and salivary proMMP-9 and -2 negatively correlated with indicators of OSAS severity ($p < 0.05$).

Conclusions: The present findings do not support a pathophysiological link between the severity of OSAS and clinical periodontal status via neutrophil enzymes or MMPs.

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1. Introduction

Obstructive sleep apnea syndrome (OSAS) is a common and treatable disorder, which involves upper airway collapse during sleep and results in intermittent hypoxaemia and sleep fragmentation. An estimated prevalence of 4% in middle-aged males and 2% in middle-aged females has been reported.[1] There is increasing evidence to support that OSAS is an independent risk factor for cerebrovascular and cardiovascular disorders including congestive heart failure, hypertension, cardiac arrhythmias, myocardial infarction, cardiac arrhythmias, and stroke.[2–4]

The apnea-hypopnea index (AHI) is used to diagnose OSAS and it is calculated as the number of apneas and hypopneas per hour during sleep. AHI score of $\geq$5 with clinical symptoms such as witnessed apneas, excessive daytime sleepiness, loud snoring and nocturnal choking or AHI $\geq$ 15 without accompanying clinical symptoms is diagnosed as OSAS.[5] An AHI score <5 is accepted within normal limits, and scores between 5–15, 15–30, and $\geq$30 indicate mild, moderate, and severe OSAS, respectively.[6] OSAS is associated with systemic inflammatory response and risk factors of periodontitis including age, gender, obesity, smoking and diabetes[7–9] are shared by OSAS. Periodontitis is also associated with systemic inflammation and has been suggested to have a role in development of cardiovascular, cerebrovascular and pulmonary diseases, as well as complications of pregnancy and diabetes.[10,11] These associations have been suggested to proceed through inflammatory pathways[12] and with systemically increased levels of inflammatory markers due to periodontal disease.[13,14] Recently published data by Seo et al.[15] provided support for an association between OSAS and periodontitis.

During inflammation neutrophils are stimulated to release their enzymes, such as matrix metalloproteinases (MMPs), neutrophil elastase (NE) and myeloperoxidase (MPO).[16] MMPs are zinc-dependent endopeptidases, known for their ability to cleave several constituents of the extracellular matrix (ECM). Zymogen forms of the MMPs (proMMPs) are secreted from a large number of cell types into the matrix and activation of the proMMPs can result in discrete alterations in tissue architecture.[17] NE is a destructive proteolytic enzyme that is increased in patients with chronic inflammation; such as chronic obstructive pulmonary disease[18] and can cause connective tissue destruction by digesting several types of ECM proteins.[19] Myeloperoxidase (MPO), another destructive enzyme expressed by neutrophils, has a role in the oxygen dependent killing mechanism of the host immune system[20] and its increased salivary levels have been reported in patients with OSAS.[21] This peroxidase enzyme is also capable of modifying acute and chronic inflammatory reactions, and activating proMMP-8, -9, and inactivating TIMP-1.[22,23] Besides these enzymes, neutrophils also contain neutrophil gelatinase associated lipocalin (NGAL), a 25-kDa secretory glycoprotein, which is widely considered as an excellent indicator of acute and chronic kidney injury and positively associated with the severity of OSAS.[17]

It is hypothesized that increased levels of inflammatory biomarkers in patients with OSAS may also affect periodontal health. Therefore, this preliminary study is undertaken to investigate serum and salivary levels of MMP-2, -8 and -9, TIMP-1, MPO, NE, NGAL, degree of activation of MMP-2 and -9 in patients with and without OSAS and to investigate whether these biochemical parameters are related with severity of OSAS and/or clinical periodontal status.

2. Materials and methods

2.1. Study population

A total of 50 patients (20 females and 30 males; age range: 21–64 years) referred to Department of Chest Disease, School of Medicine, Ege University with complaints of sleep apnea-related symptoms were included in the present study between January 2011 and September 2012. The patients were monitored overnight in-laboratory polysomnography (Compumedics E Series, Australia or Alice 5 Diagnostic Sleep System, Philips, Respironics, USA) and AHI scores were used to determine the presence and severity of OSAS.[6,24] The patients were assigned into three groups as follows; 13 patients (8 females and 5 males; age range: 21–59 years) in the control group with AHI scores <5 and diagnosed as primary snoring (non-OSAS); 17 patients (8 females and 9 males; age range: 29–64 years) in the mild-to-moderate OSAS group with AHI scores between 5 and 30, and 20 patients (4 females and 16 males; age range: 26–61 years) in the severe OSAS group with AHI scores $\geq$30. The study was approved by the Ethics Committee of Ege University, School of Medicine and conducted in full accordance with ethical principles, including the World Medical Association's Declaration of Helsinki, as revised in 2000. The aims and methods of the study were thoroughly explained and written informed consent was received from each person before their enrolment in the study.

Demographic data including age, gender, smoking status, alcohol consumption and psychotropic drug use, anthropometric measurements such as height, weight, body mass index, circumferences of neck, waist and hip, and medical histories were evaluated. Chest X-ray, arterial blood gas analysis and pulmonary function tests were also performed. Smoking status was determined by self-reporting, but smokers or former-smokers were not excluded from the study. Patients with medical disorders, such as immunological disorders, diabetes mellitus, those who received antibiotic treatment within the last 3 months, and periodontal treatment within the last 6 months, or had less than 20 teeth and those wearing removable dentures were also excluded.

2.2. Saliva and serum sampling

All patients were asked to simply expectorate into polypropylene tubes in order to collect unstimulated whole saliva samples as described previously by Navazesh.[25] Saliva sampling was performed before clinical periodontal measurements and/or any periodontal intervention, in the morning following an overnight fast during which patients were asked not to drink anything except water or chew gum. 500 μL