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Clinical and radiographic periodontal status and whole salivary cotinine, IL-1β and IL-6 levels in cigarette- and waterpipe-smokers and E-cig users

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ABSTRACT

The aim was to compare the clinical (plaque index [PI], bleeding on probing [BOP], probing pocket depth [PPD] and clinical attachment loss [CAL]) and radiographic (marginal bone loss [MBL]) periodontal parameters and whole salivary cotinine, interleukin (IL)-1β and IL-6 levels among cigarette-smokers, waterpipe-smokers, E-cig users and never-smokers. In total, 154 male individuals (39 cigarette-smokers, 40 waterpipe-smokers, 37 E-cig users and 38 never-smokers) were included. Full mouth PI, BOP, PPD and CAL were measured on all teeth (excluding third molars); and MBL was measured in digital intra-oral radiographs. Unstimulated whole salivary flow rate (UWSFR) and whole salivary cotinine, IL-1β and IL-6 levels were measured. Group comparisons were performed using one way analysis of variance and Bonferroni *post-hoc* tests. P-values less than 0.05 were considered statistically significant. There was no difference in UWSFR among the groups. Cotinine levels were significantly higher among cigarette- ($P < 0.001$) and waterpipe-smokers ($P < 0.001$) and E-cig users ($P < 0.001$) than never-smokers. IL-1β ($P < 0.01$) and IL-6 ($P < 0.01$) levels were significantly higher among cigarette- and waterpipe-smokers than E-cig users and never-smokers. There was no difference in PPD, CAL, mesial and distal MBL and whole salivary IL-1β and IL-6 levels among E-cig users and never-smokers. In conclusion, clinical and radiographic parameters of periodontal inflammation were poorer in cigarette and waterpipe smokers than E-cig users and never-smokers; and whole salivary cotinine levels were similar in all groups. Whole salivary IL-1β and IL-6 levels were higher in cigarette- and waterpipe-smokers than E-cig users and never-smokers.

1. Introduction

Clinical and radiologic examinations are usually performed for the assessment of periodontal inflammatory conditions such as periodontitis (Armitage 2004; Machtei et al. 1998). Studies (Bibars et al. 2015; Machuca et al. 2000; Natto 2005) have shown that clinical (plaque index [PI], bleeding on probing [BOP], probing pocket depth [PPD] and clinical attachment loss [CAL]) and radiographic (marginal bone loss [MBL]) parameters of periodontal inflammation are poorer in cigarette and waterpipe smokers compared with never-smokers. Literature on the impact of using electronic-cigarettes (E-cigs) on oral health particularly periodontal status is sparse; however, results from a recent cross-sectional clinical study (Javed et al. 2017b) reported no significant difference in the periodontal health status among E-cig users and never-smokers. These results should interpreted with caution as it has been shown in-vitro that E-cig liquids induce oxidative stress and increase the release of destructive-inflammatory cytokines in human gingiva cells and periodontal ligament fibroblasts (Sundar et al. 2016).

Unstimulated whole saliva (UWS) is a complex oral fluid that is collected non-invasively using simple and cost-effective techniques, such as spitting (Ghallab 2017; Kim et al. 2013). In patients with periodontitis, UWS expresses raised levels of proinflammatory biomarkers such as interleukin (IL) 1β, IL-6 and immunoglobulin G (Costa et al. 2010; Javed et al. 2009a; Javed et al. 2009b; Kaushik et al. 2011).

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Therefore, assessment of UWS is a useful investigative and screening medium. Cotinine is an alkaloid and a metabolite of nicotine, a major constituent of tobacco (Bagchi et al. 2018; Benowitz et al. 1983). The in-vivo plasma half-life of cotinine ranges from 19 to 24 h (Benowitz et al. 1983); and remains in bodily fluids including saliva, blood and urine for up to 7 days after tobacco use (Bagchi et al. 2018; Batty et al. 2018; Chen et al. 2001). Determination of cotinine levels in UWS (using enzyme linked immunosorbent assay [ELISA]) is often performed to assess nicotine exposure in tobacco smokers (Binnie et al. 2004; Kim et al. 2010; Yamamoto et al. 2005). Similarly, significantly high levels of proinflammatory cytokines such as IL-1β and IL-6 have been reported in the UWS of cigarette-smokers compared with never-smokers (Javed et al. 2015; Suzuki et al. 2016). These results suggest that cotinine and IL-1β and IL-6 are potential biomarkers for the assessment of oral inflammatory and smoking statuses. To our knowledge, there are no studies in indexed literature that have compared the whole salivary IL-1β, IL-6 and cotinine levels among cigarette- and waterpipe-smokers and E-cig users. It is therefore hypothesized that clinical and radiographic markers of periodontal inflammation and whole salivary cotinine, IL-1β and IL-6 levels are significantly higher in cigarette- and waterpipe-smokers and E-cig users, compared with never-smokers.

The aim of the present cross-sectional observational study was to compare the clinical (PI, BOP, PPD and CAL) and radiographic (MBL) periodontal inflammatory parameters and whole salivary cotinine, IL-1β and IL-6 levels among cigarette-smokers, waterpipe-smokers, E-cig users and never-smokers.

2. Material and methods

2.1. Ethical approval

The study was reviewed and approved by the Research Ethics Review Board of the College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia (CDRD 17-027). The study was performed in accordance with the Declaration of Helsinki. Participation was completely voluntary and all participants were invited to ask questions before signing the consent form. All participants were informed that there is no penalty in case they decide to withdraw from the present study at any stage of investigation.

2.2. Inclusion and exclusion criteria

Self-reported (a) cigarette-smokers; (b) waterpipe-smokers; (c) E-cig users; and (d) never-smokers were included. The exclusion criteria were as follows: (a) refusal to sign the consent form; (b) dual-smokes (individuals using at least 2 different types of smoking products, such as cigarette and waterpipe smoking or waterpipe smoking in addition to vaping); (c) E-cig users with a history of tobacco smoking; (d) self-reported smokeless-tobacco product users; (e) individuals with self-reported systemic diseases such as acquired immune deficiency syndrome, cardiovascular disorders, diabetes mellitus and renal disorders; (e) antibiotic, non-steroidal anti-inflammatory drug use and/or steroid therapy within the past 90 days; (f) lactating and/or pregnant females and (g) third molars.

2.3. Participants

Individuals that reported to be smoking at least 5 cigarettes daily for at least 12 months were defined as “cigarette-smokers” (Javed et al. 2017b). “Waterpipe smokers” were defined as individuals who reported smoking waterpipe at least once a day for a minimum duration of 12-months. “E-cig users” were defined as individuals vaping exclusively E-cigs for at least 12 months and had never used smoked tobacco in the past (Javed et al. 2017b). Individuals who reported to have never smoked tobacco and/or consumed smokeless tobacco products were defined as “Never-smokers” (Javed et al. 2007).

2.4. Questionnaire

Information regarding age, gender and duration and daily frequency of cigarette and waterpipe smoking and vaping and family history of tobacco use was collected using a questionnaire. Two trained investigators (SAM and MNA) administered the questionnaire to all participants. All participants were instructed to come during early morning hours (between 7:30 a.m. and 8:30 a.m.) in a fasting state for the collection of UWS samples; and were also advised to desist smoking and vaping overnight (approximately 10 h without nicotine exposure).

2.5. Collection of unstimulated whole saliva samples

The UWS samples were collected by one trained and calibrated investigator (AAK) (*Kappa value* = 0.88), as described elsewhere (Navazesh and Kumar 2008). In summary, participants were requested to sit motionless on a chair in a quiet room with the head leaned forward. The participants were requested to slightly open the mouth and allow UWS to drain into a funnel attached to a gauged cylinder for 5 continuous minutes. The participants were also requested to abstain from swallowing and closing the mouth throughout the procedure. The amount of saliva collected in the measuring cylinder was then measured and recorded. Unstimulated whole salivary flow rate (UWSFR) was determined by dividing the total volume of UWS collected by 5 and expressed in milliliters per minute (ml/min). All UWS samples were transferred to disposable Eppendorf tubes within 15 min of collection and frozen at −70 °C. All UWS samples were analyzed within 10 days of collection.

2.6. Assessment of whole salivary cotinine and IL-1β and IL-6 levels

Levels of cotinine in UWS were measured using ELISA. The 96-well ELISA plates (Nunc A/S, Roskilde, Denmark) were overnight coated (0.1 ml/well) with an anti-goat IgG (10 μg/ml) (Dako Cytomation A/S, Glostrup, Denmark) in tris-buffer (pH 8.4). Using phosphate buffer (10 mM; pH 7.5), the plates were blocked and incubated for 1 h at room temperature. Serial dilutions of a solution consisting of cotinine (160 ng/ml) in phosphate-buffer were used to generate a standard curve. Each sample (50 μl of 0.01% goat polyclonal anti-cotinine reagent) (Affiniti Research Product Ltd, Exeter, UK) was assessed in duplicate wells and 50 μl of cotinine (Aldrich Chem Co., Milw., WI, USA) conjugated with horseradish peroxidase (Sigma Co., St Louis, MO, USA). The plates were incubated for 1 h at 25 °C, and washed 3 times with distilled water. A substrate solution consisting of 100 μl tetramethylbenzidine was then added, and plates were incubated for 30 min at 25 °C in a dark room. Phosphoric acid (100 μl; 1 M) was used to terminate color development and the optical density of each well was determined at 450 nm using a microplate reader (Microplate Reader 3550, Bio-Rad, CA, USA). This technique is described elsewhere (Matsumoto et al. 2010). The UWS samples were collected by one trained and calibrated investigator (AAK) (*Kappa value* = 0.86).

To determine whole salivary IL-6 (R&D Systems Inc., Human interleukin-6 Quantikine, ELISA Kit, Minneapolis, MN., USA) and IL-1β (SALIMETRICS LLC, Salivary interleukin 1 beta, Carlsbad, CA., USA) levels, ELISA kits were used according to the manufacturers’ instructions. One hundred microliters of diluted standards with UWS samples were dispensed (in duplicates), into the 96-wells ELISA plated that were precoated with a specific protein antibody. The plates were incubated at room temperature for one hour and then washed 3 times. A conjugate solution (100 μL) was then added, and the plates were again incubated at room temperature for 2 h. After washing the trays 3 times using a wash solution, a substrate solution (100 μl) was added. The plates were reincubated for 30 min at room temperature; and 50 μl of stop solution was added to stop color development. Using a microplate reader (Microplate Reader 3550, Bio-Rad, CA, USA), absorbance was assessed by reading the plate at 450 nm. Using standards provided with the IL-6

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