



ORIGINAL ARTICLE

Correlation between the extent of smoking, salivary protein profiles, and dental caries in young adult smokers



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KEYWORDS

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Molecular weight;
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Abstract Context.

Proteins in the saliva are one of the defense mechanism factors that can protect the oral cavity from disease. However, smoking might affect the properties of saliva.

Aim: To determine the differences in salivary protein profiles and total concentrations in smokers and non-smokers and their correlation with dental caries severity as indicated by the Decayed, Missing, Filled-Teeth (DMF-T) scores.

Methods and material: This cross-sectional study included 25 smokers and 25 non-smokers. The DMF-T scores were recorded. The total salivary protein was measured by the Bradford method, and the profile proteins were determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).

Results: The average of salivary protein concentration in smokers was lower than that in non-smokers (551.486 µg/mL versus 765.361 µg/mL), but the difference was not statistically significant ($P > 0.05$). Further correlation analyses showed a negative correlation between the concentration of proteins based on the extent of smoking. A weak negative correlation was found between protein concentration and DMF-T scores ($r = -0.239$). Dominant salivary protein bands of 11.6 kDa and 54.5 kDa were found in smokers and 27 kDa, 60 kDa, and 94.5 kDa were found in non-smokers.

Conclusion: Different protein bands appeared in smokers and non-smokers. There was a weak correlation between protein concentration, DMF-T scores, and the extent of smoking.

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1. Introduction

Smoking has various adverse effects on health, one of which is the predisposition to pathological conditions in the oral cavity (Feifei, 2017; Pasupathi et al., 2009). Cigarette addiction can result in a decreased quality of life. Previous studies have

reported that cigarette components that enter the smoker's body through cigarette smoke can affect the body's defense system (Qiu et al., 2017; Sopori, 2002).

Saliva is a protective fluid in the oral cavity that is in contact with cigarette smoke (Khan et al., 2010; Kolte et al., 2012). Saliva has several important components, one of which is protein. Proteins that preserve the body's immunity serve as potential biomarkers to monitor pathological conditions. Hence, saliva can be used to measure the status of dental and oral health in a person (de Almeida et al., 2008; Martins, 2013; Khan et al., 2010; Ramalingam et al., 2013; Streckfus, 2015).

Caries is a complex multifactorial disease characterized by the loss of mineral ions on the tooth surface (Lenander-Lumikari and Loimaranta, 2000; Naskova et al., 2016). The DMF index is applied to the permanent dentition and is expressed as the total number of teeth or surfaces that is decayed (D), missing (M), or filled (F) in an individual. The DMFT index is a fixed index that can measure the prevalence or number of caries events in a person or a population (Kolte et al., 2012). Our previous study reported a relationship between total salivary protein concentration and the DMFT index (Bachtiar et al., 2017). Here, we further examined the differences in salivary protein profiles and total concentrations and their association with caries formation as measured by the DMF-T index score.

2. Material and methods

This cross-sectional study included 50 saliva samples from young adult students, aged 18–24 years. The study population consisted of 25 smokers and 25 non-smokers (as controls). A consecutive sampling method was used, with the following inclusion criteria: good health, absence of systemic disease or history of systemic disease, and normal growth and development. The exclusion criteria were: systemic or congenital diseases related to the oral cavity, ongoing medical therapy, and current intake of local or systemic medication. The smokers were classified into "light," "moderate," and "heavy" smokers based on the number of cigarettes consumed in a day (light: 1–10 cigarettes, moderate: 11–20 cigarettes, and heavy smoker: > 20 cigarettes). We obtained ethical approval for this study (No: 89 / Ethics Agreement/FKGUI / VII / 2018).

2.1. Sampling and sample preparation

The samples used 1.5 mL of unstimulated saliva, which was collected using a sterile pipette and stored in a 1.5 mL falcon cup containing phenylmethylsulfonyl fluoride (PMSF) solution. The samples were centrifuged at temperature of 4 °C for 30 min at a speed of 4000 rpm to separate saliva from the supernatant and pellets. The salivary supernatant was then transferred to another falcon cup.

2.2. Protein assay

The Bradford method measured the protein concentration. Bovine serum albumin was used as a standard protein and saliva samples were added to the well plate at a concentration of 10 µL; 190 µL of Coomassie Brilliant Blue reagent was added

in each well and then read by a microplate reader/ELISA reader based on the optical density (OD) at a wavelength of 595 nm.

2.3. SDS-PAGE

The SDS PAGE method was carried out by mixing 20 µL of a native buffer sample with 20 µL of a saliva sample and then heating the mixture in a thermal block at 98 °C for 10 min. Stacking gel and resolving gel were made until a well was formed on the stacking gel. Fifteen microliters of samples and 5 µL of protein markers (SMOBiO PM2700) were placed in each well of the stacking gel for electrophoresis. Electrophoresis was performed at 80 mA, with a voltage of 150 V for 60 min. After electrophoresis, the gel was stained with a Coomassie Blue dye and left for one night on a shaker at a speed of 60 rpm. Then, the gel was destained on a shaker at a speed of 60 rpm for 30 min. The gels were analyzed through a scanning process using a scanner to determine the molecular weight of the proteins from protein bands that appeared in the gel with a quick band analysis guide (Bachtiar et al., 2016).

3. Results

Fig. 1 and Fig. 2 show the concentration of salivary proteins in smokers and non-smokers. The total protein concentration in the smoker group was 551,486 µg/mL, lower than the total protein concentration in the non-smoker group (765,361 µg/mL). The percentage difference in total salivary protein concentration between the two groups was 29.05%. However, statistical tests indicated that the difference was not significant ($P > 0.05$).

In this study, the smokers were categorized according to the WHO recommendations, which is based on the number of cigarettes consumed in a day. Fig. 2 shows that the salivary protein concentration in the three categories of smokers was lower than that in the non-smoker group. However, the difference in total salivary protein concentration between the mild smokers and non-smokers was not significant ($P > 0.05$). On the other hand, the salivary protein concentrations in the moderate and heavy smoker categories were significantly lower (411.56 µg/mL (46.3%) and 337.4 µg/mL (56%), respectively), than the total salivary protein concentration of the non-smokers group.

DMF-T is an index used to measure the incidence or caries experience of an individual or population. Fig. 3 shows the

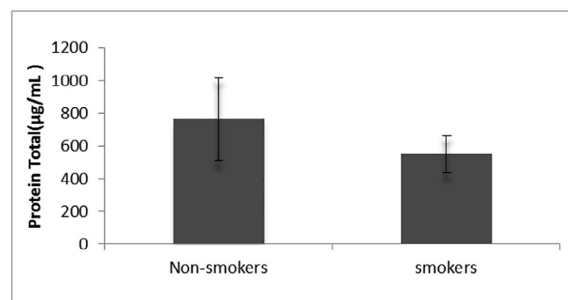


Fig. 1 The total salivary protein concentration in smokers and non-smokers.

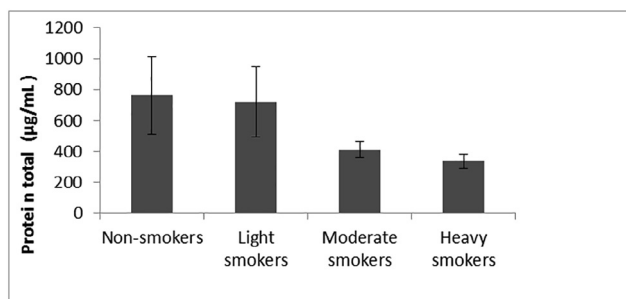


Fig. 2 The total protein concentration in non-smokers, based on the extent of smoking.

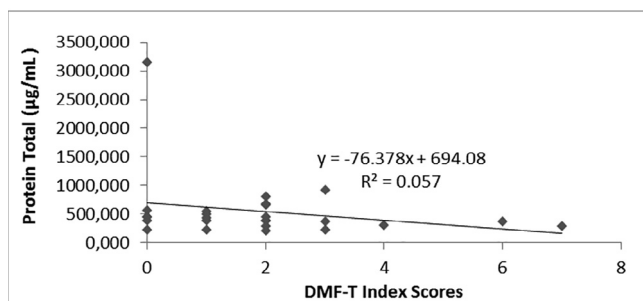


Fig. 3 The correlation between the total salivary protein concentration and DMF-T scores in the smoker subjects.

results of the correlation analysis. Pearson's correlation coefficient indicates a negative correlation between the total concentration of salivary proteins and DMF-T index scores of smokers ($r = -0.057$). However, this correlation was not significant ($P > 0.05$).

There were differences in salivary protein profiles between smokers and non-smokers, as shown in Fig. 4. Representative salivary protein bands in SDS-PAGE from smokers and non-smokers. The sample of smokers showed bands of 11.6 kDa and 55 kDa. The protein bands of 27, 60, and 94.5 kDa were found in the sample of non-smokers. Proteins with a molecular weight of 11.6 kDa and 55 kDa were found more frequently in smokers than in non-smokers. Both proteins were dominant proteins that appeared on the SDS-PAGE gel of smokers (Fig. 5). In non-smokers, dominant proteins were found with molecular weights of 27, 60, and 94.5 kDa. These results indicate a difference in salivary protein profiles between smokers and non-smokers. No appearance of proteins with molecular weights of 27, 60, and 94.5 kDa on SDS-PAGE gel in the smoker group (Bachtiar et al., 2016).

4. Discussion

This study found that salivary protein concentrations were lower in smokers than in non-smokers, although the differences were not statistically significant. Our research results were similar to those reported in a previous study (Kolte et al., 2012). This phenomenon may be the result of the nicotine component that can inhibit the sympathetic nerve ganglion and result in reduced production of saliva and its components (Kolte et al., 2012; Pasupathi et al., 2009; Qiu et al., 2017). Another study reported that the salivary protein

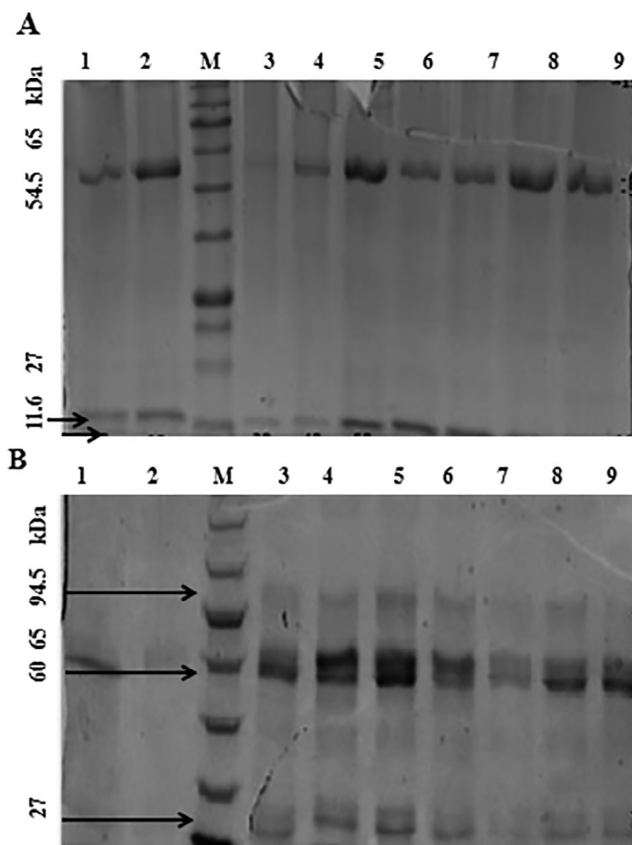


Fig. 4 The representative salivary proteins' bands found through SDS-PAGE of the samples of smokers and non-smokers. A. The sample of smokers showed the bands of 11.6 kDa and 55 kDa. B. The protein bands of 27, 60, and 94.5 kDa were found in the sample of non-smokers.

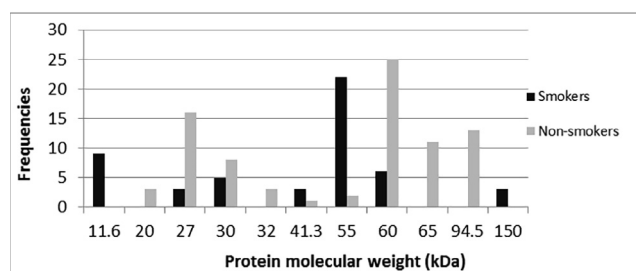


Fig. 5 The frequency of appearance of salivary proteins' protein bands in the sample of smokers and non-smokers.

concentration was lower in smokers because the chemical composition of cigarettes affects the quantity and quality of saliva, which reduces its protein component, which is essential for the defense system in the oral cavity (Pavitra et al., 2013; Khan et al., 2010; Ramalingam et al., 2013). Our study revealed a lower total protein concentration in smokers, and protein bands that we found in the non-smoker group were not found in the smoker group.

In this study, the total salivary protein concentrations in the light to the heavy smoker categories were lower than in the non-smoker group. However, a significant difference in total salivary protein concentration in the smoker group was only

found in the moderate and severe categories. The frequency of cigarette consumption affects the quantity and quality of saliva. A previous study reported that the higher the rate of cigarette consumption, the worse the total protein concentration in saliva (Streckfus, 2015). In this study, the total protein concentration in the heavy smokers category was lower than that in the light and moderate smoker categories.

Salivary protein level is correlated with caries because it is a defense mechanism in the oral cavity that can interact with oral cavity bacteria by limiting bacterial growth and development, and by interfering with bacterial glucose uptake or glucose metabolism (Naskova et al., 2016). The DMF-T index score is a measurement used to determine dental health status, to assess the experience or severity of dental caries (Huang, 2004). In this study, there was a weak negative correlation between total salivary protein concentration and DMF-T index scores. According to the study by Naskova et al. (2016), there was a negative correlation between total salivary protein concentrations and DMF-T. The study reported that a decrease in total salivary protein was associated with an increase in cariogenic bacterial colonization, which produced acidic products that predisposed to caries formation and caused an increase in the DMF-T index scores (Naskova et al., 2016).

In addition to comparing the total salivary protein concentrations in smokers and non-smokers, this study also aimed to determine salivary protein profiles in smokers. The results of this study indicated that there were differences in protein profiles between smokers and non-smokers, based on the molecular weight (kDa).

The dominant salivary protein bands, with molecular weights of 11.6 kDa and 54.5 kDa, were found in smokers. The 11.6 kDa molecular weight protein was suspected to be thioredoxin (Collet and Messens, 2010; Huang, 2004). Thioredoxin is an antioxidant protein found in saliva that can protect cells from various free radicals produced from cigarette smoke (Huang, 2004; Collet, 2010). According to the study by Huang, the 54.5 kDa molecular weight protein was suspected to be an enzyme called catalase (Huang, 2004). This enzyme includes the hydrogen peroxidase enzyme, which protects the body against dangerous peroxide compounds that can produce free radicals (Collet, 2010). In this study, both proteins were found at a high frequency in the smoker group. Their study indicated that cigarette components are toxic to mammalian cells, so thioredoxin and catalase are needed to prevent the toxic effects (Bik et al., 2010).

On the other hand, there were dominant salivary protein profiles in non-smokers with molecular weights of 27 kDa, 60 kDa, and 94.5 kDa. According to the study by Huang (2004), the 27 kDa molecular weight protein was suspected to be a proline-rich protein (PRP). PRPs play a role in inhibiting demineralization and preventing calculus formation. This protein maintains Ca^{2+} levels in the saliva constant, thereby improving remineralization (Bhalla et al., 2010; Bik, 2010; Rudney et al., 2009).

Proteins with a molecular weight of 60 kDa are α -amylase proteins, which are the most abundant proteins and enzymes in saliva (Amado et al., 2005; Rudney et al., 2009). In this study, smokers had lower amylase levels. According to Fujinami et al., cigarettes can reduce the function of several proteins, including salivary amylase, which acts as an antibacterial agent by inhibiting the growth and attachment of some bacteria (Amado et al., 2005; Pendyala, 2013;

Sopori, 2002). Amylase is a carboxylase enzyme that digests carbohydrates into simple sugars. Reducing carbohydrates will inhibit bacterial growth (Amado et al., 2005, 2013).

The 94.5 kDa molecular weight protein was suspected to be a secretory component in saliva, namely s-IgA (Huang, 2004). S-IgA is the primary immunoglobulin molecule of saliva that acts as an antimicrobial defense system. This study found that in the smokers' group, there was a decrease in s-IgA levels, which occurred because the chemical component in cigarettes can reduce the level of s-IgA (Sopori, 2002; Pendyala et al., 2013). Previous studies reported that s-IgA is more often detected in caries-free subjects, and the immunoglobulin plays a role in the defense system in the mouth by inhibiting bacterial colonization (Fattahi et al., 2015; Qiu et al., 2017). The results of this study indicated that various dominant proteins identified in the smokers' group had a function to support homeostasis in the oral cavity, especially in preventing caries formation.

This study also had some limitations. We only used the saliva samples of young adults, i.e. students aged 18–24 years. As such, the conclusions of this study cannot be generalized to people of all ages. Also, further research is needed to identify the protein bands by western blotting.

5. Conclusion

The total salivary protein concentrations were lower in smokers than in non-smokers, and there were differences in salivary protein profiles between smokers and non-smokers. In addition, there was a weak negative correlation between salivary concentration and the occurrence of dental caries.

Further research to identify the 11.6 kDa and 54.5 kDa proteins that were found in smokers and the 27 kDa, 60 kDa, and 94.5 kDa proteins found in non-smokers by western blotting is needed.

This study may stimulate further research, especially in finding strategies to improve oral health among smokers.

Ethical statement

This study 'Correlation between the extent of smoking, salivary protein profiles and dental caries in young adult smokers' obtained the ethical approval from the Ethical Research Committee Faculty of Dentistry University of Indonesia (No: 89/Ethics Agreement/FKGUI/VII/2018).

CRedit authorship contribution statement

Endang W. Bachtiar: Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Resources, Software, Visualization. **Destri S. Gusliana:** Data curation, Formal analysis, Investigation, Writing - original draft. **Boy M. Bachtiar:** Conceptualization, Data curation, Resources, Supervision, Validation, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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