ORIGINAL ARTICLE

How Does Smoking Alter Saliva Components When Exposed to Panoramic Radiography?

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ABSTRACT

Introduction: Cigarette smoke harbors over 7,000 chemical agents, thereby subjecting smokers to a multitude of toxins. Smoking induces oxidative stress indicated by increased oxidative damage of biomarkers on protein, DNA, and lipid. Panoramic radiography is one type of radiography technique where x-ray beams pass through the salivary gland, which makes it receive the highest absorbed doses compared to other organs and thus can affect the acinar cells. **Materials and methods:** Unstimulated whole saliva samples were taken three times: before exposure (E1), right after exposure (E2), and ten days after exposure (E3). Salivary pH was measured with the LAQUAtwin pH-11 Horiba pH meter. Buffer capacity was determined using the Ericsson method, while total protein, sodium, and potassium levels were calculated with the Kyltec autoanalyzer. Statistical analysis was done with the computerized statistical test, using a nonparametric test Kolmogorov Smirnov and a comparative test oneway Anova. **Results:** The result of saliva components tested on this study showed a lower value for all components in smoker compared to non smoker. There were significant differences between salivary pH, $p<0.05$ in both groups, and total protein level in the smoker group ($p<0.05$) (Table I). In contrast, other components in both groups had no significant difference after being exposed to panoramic radiograph. **Conclusion:** This study found that panoramic radiographs significantly affect salivary pH before and after exposure in both groups, as well as the total protein level in the smoker group. However, other tested salivary components showed no significant difference in all time intervals.

Keywords: Buffer capacity, Panoramic radiograph, Potassium level, Saliva pH, Sodium level, Total protein

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INTRODUCTION

Smoking is one of the most common causes of health problems worldwide. Indonesia is renowned as a nation with a significantly elevated prevalence of smoking habits.(1) Based on Basic Health Survey, 2010 (34.7%), 2013 (36.3%), 2018 (39.1%), and 2021 (41.1%) showed an increasing trend of smoking prevalence throughout the year (1,2).

Tobacco use can cause severe addiction syndrome, and it has been widely proven that smoking has a detrimental effect on the entire organs of the human body. Cigarette smoke is comprised of over 7,000 chemical agents, exposing smokers to a wide range of toxins (3). These toxins include carbon monoxide, nitrogen, nicotine, and free radicals such as superoxide, hydroxyl, hydrogen peroxide, and reactive oxygen species (3). Smoking induces oxidative stress indicated by increased oxidative damage of biomarkers on protein, DNA, and lipid (4,5). Antioxidants contained in saliva, including enzymes, urea acid, and glutathione able to defend saliva against free radicals but salivary antioxidant activity in smokers can not protect against cumulative stressors of cigarette smoke. Absorbed nicotine from cigarettes is distributed in very high amounts to several glands, such as the salivary and adrenal glands. High doses of nicotine can induce pathological processes in salivary glands through morphological changes in myoepithelial cells, disrupting the glands' function (6,7).

Panoramic radiography is one type of extraoral radiography technique commonly used in the field of dentistry because it provides a complete anatomical view of a facial structure, including teeth, maxillary and mandibular dental arch, temporomandibular joints, supporting structures, and even some anatomical variations in one single image (8). In panoramic radiography, x-ray beams pass through the salivary gland, which makes it receive the highest absorbed doses compared to other organs and thus can affect the acinar cells. Radiation exposure can induce ionization reactions that will produce free radicals, which have the

capability to disrupt DNA and cell apoptosis of acinar cells. 20 Gy radiation exposure on oral mucous can cause clinically visible redness due to cell apoptosis. Exposure to ionizing radiation, even at low levels, has the potential to inflict direct harm to DNA structures. It instigates the production of reactive oxygen species and free radicals that in turn cause detrimental effects on DNA, proteins, and lipid membranes (9). This effect is known as stochastic effects that do not have a threshold dose of radiation. Based on this background, the authors were interested in studying the saliva composition in smokers that undergo panoramic radiography assessment (10).

MATERIALS AND METHODS

Study design

This study was quasi-experimental with a pre and post-test-only control group design conducted in the Dental Hospital Universitas Hasanuddin and Oral Biology Laboratory, Faculty of Dentistry, Universitas Hasanuddin. All patients who participated in this study were patients referred to the Department of Dental Radiology to have panoramic radiographs and signed informed consent. Ethical clearance and feasibility were obtained from the Research Ethical Committee, Faculty of Dentistry, Universitas Hasanuddin [Reference No; 0128/E2.08/KEPKFKG-RSGM UNHAS/2020], in accordance with the Helsinki Declaration.

Populations

The inclusion criteria of the study samples from the smoking patients population were having smoking habits minimum of five years, having no history of systemic or autoimmune disease, not using any dental prostheses, not being under any type of medication for more than three months, not consuming any alcoholic drinks, and not undergo any radiographic assessment 15 days prior to the study. As for the samples from non-smoking population, the inclusion criteria were similar to the smoking population except for the smoking habits and several additional measures such as good oral hygiene, normal oral mucosa, and no lesion or ulcer in the oral mucosa. Fifty male patients were included in this study, divided into two study groups, 25 non-smoking and 25 smoking patients. They were interviewed and asked for informed consent as an agreement to participate in this study.

Saliva sampling

Unstimulated whole saliva samples were taken three times before radiographic exposure (E1), right after exposure (E2), and ten days after exposure (E3) using the passive drooling method, as it is considered to be a promising way to minimize the potential sources of error. All samples were taken in the morning to prevent bias induced by circadian and circannual rhythms. Patients were restricted to having food and drink before saliva collection and instructed to rinse with deionized water and wait 30 minutes before starting the collection. Saliva samples were collected into sterile tubes for as many as 5 mL of each patient, transferred to the lab, frozen, and stored at -80°C until analyzed.

Saliva analysis

Salivary pH was measured with the LAQUAtwin pH-11 Horiba ph meter. The determination of buffer capacity was conducted utilizing the Ericsson method. In this procedure, 0.5 mL of saliva was combined with 1.5 mL of a solution containing a concentration of 5 mmol/l. Subsequently, the mixture underwent shaking and centrifuging for one minute, followed by a resting period of 10 minutes. Finally, the pH value of the resulting solution was measured using a pH meter.(11) Total protein, sodium and potassium levels were calculated with kyltec autoanalyzer.

Statistical analysis

All statistical analysis were performed with the computerized statistical test, using a nonparametric test Kolmogorov Smirnov and a comparative test oneway Anova. The significance level was set at 0.05.

RESULTS

The result of saliva components tested on this study showed a lower value for all components in smoker group compared to non smoker group in Figure 1-5.

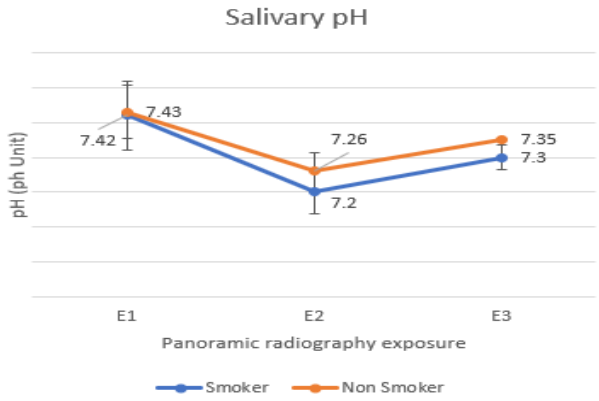

Figure 1: Salivary pH on both groups on all time intervals.

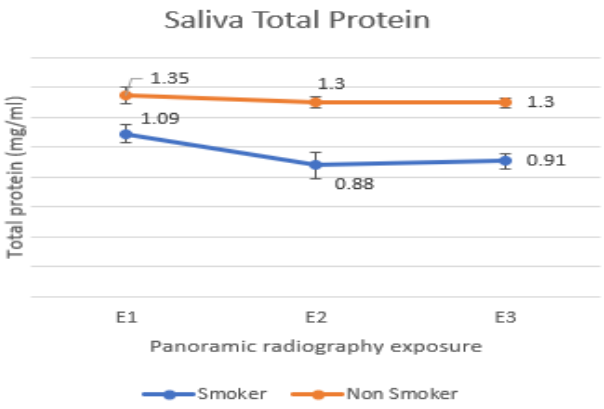

Figure 2: Saliva total protein on both groups on all time intervals.

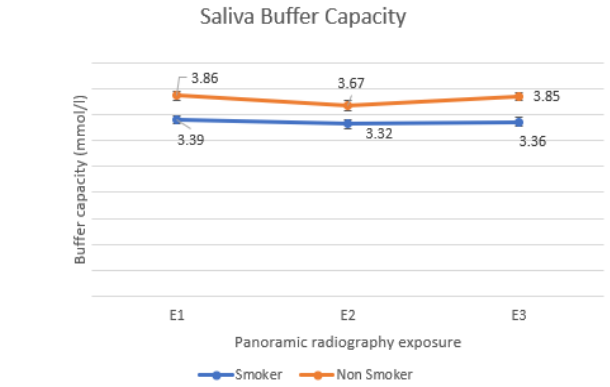

Figure 3:Saliva buffer capacity on both groups on all time intervals.

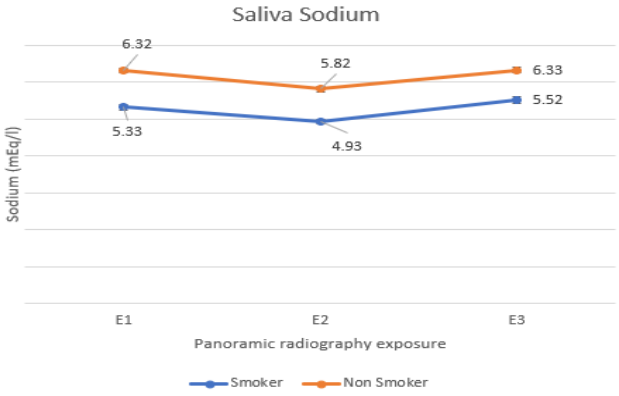

Figure 4: Saliva sodium level on both groups on all time intervals.

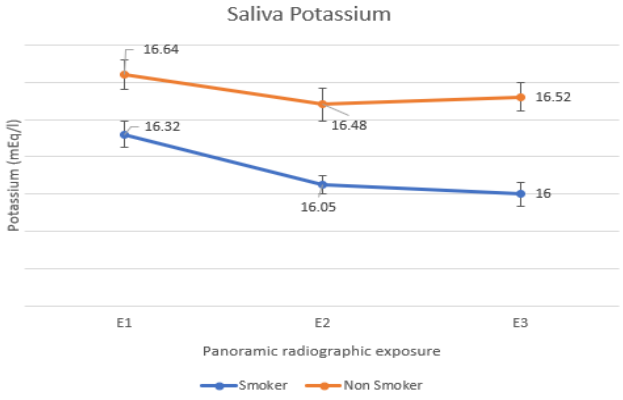

Figure 5: Saliva potassium level on both groups on all time intervals.

There were significant differences of salivary pH in smoker and non smoker group, p<0.05 in both groups, and total protein level in smoker group (p<0.05) (Table I) while for other components in both groups have no significant difference after exposed to panoramic radiograph.

Table I: Saliva component analysis on both groups after exposed to panoramic radiography.

Group	Time interval	Non- Smoker	Smoker
Saliva components		p value	p value
pH	E1-E3	0.004*	0.001*
Total protein	E1- E3	0.721	0.029*
Buffer capacity	E1-E3	0.358	0.936
Sodium	E1- E3	0.576	0.229
Potassium	E1-E3	0.960	0.610

*p<0.05 = statistically significant

Salivary pH on smoker and non smoker group (Figure 1) showed a lower value right after exposure (E2) but increase on ten days after exposure (E3). However, the pH value ten days after exposure is not the same as before exposure (E1). The same thing applies to total protein (Figure 2) where total protein value right after exposure (E2) was lower than before exposure (E1). But the total protein value ten days after exposure (E3) is almost similar with before exposure (E1) in non smoker group, while in smoker group the total protein value ten days after exposure (E3) was still lower than before exposure (E1).

However, in other tested saliva components, such as buffer capacity and sodium (Figure 4 and Figure 5), the value was lower right after exposure (E2) but the value ten days after exposure (E3) is almost similar to the value before exposure (E1).

DISCUSSION

This study showed a lower salivary components on smoker group than non smoker group even before the exposure of panoramic radiograph (E1). Saliva have a pivotal role in oral cavity homeostasis, whereas any alterations in salivary secretion may lead to a number of adverse effects. There are several studies discussing the effect of nicotine to salivary components but only a few adressed its effect along with the exposure of panoramic radiograph.

Previous research by Hasyim et al on salivary component after exposure of intraoral radiograph showed that there were no significant effects on salivary pH, total protein, buffer capacity, sodium, and potassium level on the five time intervals tested (12). The use of panoramic radiography in this study involves the utilization of X-rays, which fall under the category of electromagnetic rays. It is important to note that exposure to radiation can have biological effects on the body. These effects can occur both directly and indirectly. When ionizing radiation interacts with matter, it initially occurs at the electron level within the first 10-13 seconds after exposure. Approximately two thirds of biologic damage caused by radiation is a result of indirect effects, while one third is attributed to direct effects. The impacts of these biological processes can manifest as transient disruptions in the functionality of endothelial cells (13). When exposed to X-rays, blood vessels undergo brief vasoconstriction followed by vasodilation, consequently altering blood vessel permeability. Saliva's water components and bicarbonate ions originate from the interstitial fluid and blood plasma found within the capillary blood vessels surrounding the salivary glands. The absorption of ionizing radiation by biological molecules leads to direct effects, resulting in the formation of unstable free radicals (14). This occurs within a very short timeframe, less than 10-10 seconds, after the interaction with a photon (14). Given that water

is a major component, comprising about 70% of biologic systems, it often plays a role in the interactions between x-ray photons and biologic components (14,15).

A study by Nurgalih et al found a decreased salivary pH after exposed to panoramic radiograph (6.91 before exposed, and 6.82 after exposed) (16). This is in line with a study conducted by Susanti et al where salivary pH decreased from 7.8 to 7.7 after panoramic radiograph exposure. The variance in salivary pH levels observed in this study may be attributed to radiation exposure from panoramic radiography. It is important to note that even small doses of radiation from panoramic radiography can impact exposed cells and tissues (16). The effective dose emitted during panoramic radiography surpasses the maximum allowable effective dose.

This study found that smokers had significantly lower levels of total protein compared to non-smokers, with a significant difference in smoker group ($p<0.05$). This decrease can be attributed to the fact that most proteins are secreted by acinar cells, and any impairment in their activity leads to a decrease in the secretion of total proteins. Moreover, tobacco-related toxic products can cause injury to the acinar and ductal secretory unit, further contributing to the reduction in total proteins. As a result, smokers also experience decreased levels of immunoglobulin and enzymes that work on saliva, as well as a decrease in glutathione which serves as the main antioxidant in the mouth (17,18). Differences in the data of salivary pH and buffer capacity found in this study may be attributed to the three essential salivary buffer systems - carbonate, phosphate and protein buffers. The concentration levels of these buffers and their respective buffering capabilities are closely linked with the rate at which saliva is generated (19).

The release of free electrons during x-ray radiation exposes cellular components, like water, to undergo the formation of free radicals. These radicals result in the production of H2O2, which leads to apoptosis specifically in serous asini cells within the salivary gland. As serous asini cells are more susceptible to radioactivity than mucous asini cells, the saliva becomes thicker and stickier. The primary impact of radiation on the salivary gland is damage to the plasma membrane of acinar cells, subsequently affecting muscarinic receptors responsible for stimulating salivary secretion (20).

This study found that there were no significant decrease in sodium and potassium level on both group after exposed to panoramic radiograph. A study conducted by Lan et al showed a decrease concentration of saliva electrolyte on patient received radiotherapy treatment, which could be resulted by the destruction of plasma membran and corresponding ion channel in acinar cells due to high doses radiation (21). The extent of adverse effects caused by radiation is dependent on the radiation doses received. As a result, the varying levels of radiation doses have a significant impact on changes in saliva component (22).

There are limited research regarding exposure to low doses of radiation and its effect on saliva, hence only a few studies that can be used as a comparison of the results. Nonetheless, the result of this study that found a significant effect on salivary pH in both groups and total protein in smoker group can become a consideration in clinical settings when the patient have to undergo a repeat radiographic exposure.

CONCLUSION

This study found that panoramic radiographs have a significant effect on salivary pH before and after exposure in both groups, as well as total protein level in the smoker group. However, other tested salivary components showed no significant difference in all time intervals.

ACKNOWLEDGEMENT

The authors wish to acknowledge the staff members of Department of Oral and Maxillofacial Radiology, Hasanuddin University Dental Hospital for their support throughout this study.

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