

# Analysis of genotoxic and cytotoxic effects of oral mucosa in smokers and non-smokers after exposed to digital intraoral radiography



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## Abstract

**Objective:** To analyze genotoxic and sitotoxic indicators of buccal epithelial cells by measuring the number of cells contain micronucleus, pycnosis, karyorrhesis, karyolysis and to determine the change in oxidative stress of oral mucosa cells using salivary MDA levels between smokers and nonsmokers before and after exposure to intraoral radiographs during endodontic treatment.

**Material and Methods:** This research was an analytic observation research with cross-sectional design. The sample was divided into smokers (n=5) and non-smokers group (n=5). The buccal epithelial cells was taken with cytobrush and unstimulated saliva in each group were taken by the draining method shortly before intraoral radiography, 10±2 days after first day exposure, 10±2 days after second day exposure. The cells were stained using Papanicolau (PAP) kit and observed by pathologist. Genotoxic and cytotoxic indicator

measured by counted the number of cells contain mironucleus, pyknosis, karyorrhesis and karyolysis under light microscope with 400X magnification according to the Tolbert criteria in 1000 cells. While oxidative stress of cell measured by salivary MDA with Thiobarbitoric Acid (TBA) assay.

**Results:** The number of micronucleus, pyknosis, karyorrhesis, karyolysis in buccal epithelial cells and salivary MDA levels in smokers was higher than non-smokers (p<0.05). There were no significant differences in genotoxic and cytotoxic indicators and salivary MDA levels before and after the last exposure in smokers and non-smokers (p>0.05).

**Conclusion:** The results of this study indicate that the use of intraoral radiographs is within safe limits despite repeated exposure after several days.

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## Introduction

Intraoral radiographic examination has been widely used to complemet physical assesment in dental treatment. Although the radiation dose in intraoral examination given in a small dose, it still has a radiobiological effect on exposed tissue. One of the causes of cell or tissue damage is due to the occurrence of oxidative stress by free radicals.<sup>1</sup> Biological systems can be exposed to free radicals either formed endogenously by the body's metabolic processes or exogenous as well as the effects of cigarette smoke exposure. Free radicals are highly reactive and can lead to biochemical changes and damage various components of living cells such as proteins, lipids, carbohydrates and nucleates. The cell membrane consists mainly of lipid components. The attack of free radicals on the lipid component would lead to lipid peroxidation reactions and then produce products that are toxic to cells.<sup>2,3</sup>

Cigarette smoke contains large quantities of free radicals and more than 7.000 chemicals. Smoking would increase the biomarkers of oxidative damage to proteins, DNA and lipids.<sup>4,5</sup> In addition, free

radicals in cigarette smoke also cause changes in quality and quantity of saliva.<sup>6</sup>

The buccal mucosa is the main barrier in oral cavity and buccal epithelial cells can reflect cells affected by genotoxic induced by carcinogenic agents through the formation of micronucleus.<sup>7</sup> While cytotoxic can reflected in buccal epithelial cells through the formation of pycnosis, karyorrhesis and karyolysis.<sup>8,9</sup> Saliva is widely used in clinical research to detect diseases in the oral cavity. There are a lot of literature that discusses the use of saliva as an alternative biological sample to determine the diagnosis, prognosis and management of oral diseases with salivary MDA as a marker.<sup>6,10</sup>

Salivary malondialdehyde (MDA) is formed from lipid peroxidation on cell membranes by free radical reactions (hydroxy radicals) with Poly Unsaturated Fatty Acid (PUFA). The reaction occurs in a chain reactions, the final result of the chain reaction is hydrogen peroxide.<sup>11</sup> Hydrogen peroxide can lead to decomposition of some aldehyde products that are toxic to cells and differ in

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length, including MDA, which is one of the main aldehydes formed. In high oxidative stress conditions, there is a significant increase in MDA levels. If the condition of oxidative stress is resolved, MDA levels returns to the normal condition.<sup>12</sup>

This study was conducted to analyze differences of cells contain micronucleus, pycnosis, karyorrhesis, karyolysis and salivary MDA levels in smokers and nonsmokers before and after exposed to intraoral radiographs in patients undergoing endodontic treatment.

## Material and Methods

This study was a cross sectional study conducted at the Department of Dental Radiology, Dental Hospital of Hasanuddin University. Institutional ethical committee approval (registration number: UH 17120133) and written informed consent from all the samples were obtained before sampling. The number of samples in this study were 10 people divided into smokers group (n=5) and non smokers (n=5).

Non-smokers samples were those who fulfilled the inclusion criteria: did not smoke, did not

consume alcoholic drinks and did not undergo any radiographic examinations in the past 6 months. Inclusion criteria for smokers were: have a smoking habit every day for at least the last three years, did not consume alcoholic drinks and did not undergo any radiographic examinations in the past 6 months.

Samples were taken based on radiographs schedule of endodontic treatment; (P0) Before exposure, (P1) 10±2 after 1<sup>st</sup> and 2<sup>nd</sup> exposure (1<sup>st</sup> day exposure with effective dose 0,008 mSv/day), (P3) 10±2 after 3<sup>rd</sup> and 4<sup>th</sup> exposure (2<sup>nd</sup> day exposure with effective dose 0,008 mSv/day). In the initial cell and saliva collection, the patient was instructed to rinse their mouth three times with provided rinse water. Saliva was taken with draining method. Patients were asked to sit with their heads down and mouth open to allow saliva flow passively from the lower lip to sterilized calibrated test tubes. Saliva was collected without any stimulation. The test tubes then labeled according to the sample's data and stored in the cooler box and immediately taken to the laboratory for analysis with Thiobarbitoric Acid (TBA) assay. Exfoliated buccal epithelial cells was taken by rotating cytobrush on the buccal mucosa surface (inner cheek) surrounding the exposed teeth with a one-time clockwise circular motion of 360°. Cytobrush smears were prepared on cleaned microscopic slides and allowed to air dry and then fixed in alcohol 96%. The slides were stained using Papanicolau (PAP) method and examined under Olympus light microscope at 400X magnification and number of micronucleus were scored from 1000 cells used criteria by Tolbert et al.<sup>13</sup> Intraoral radiograph using Belmont PHOT-XII 303 (70 kVp, 10mA, 3sec) with effective dose 0,004 mSv.

## Results

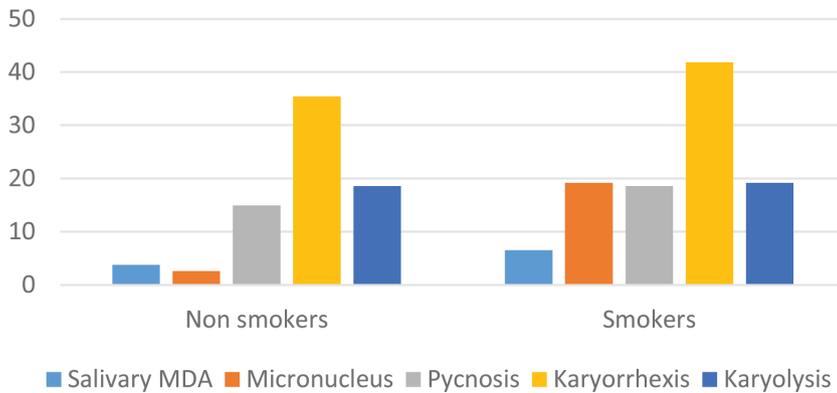
The number of micronucleus, pyknosis, karyorrhesis, karyolysis in buccal epithelial cells and salivary MDA levels in smokers was higher than non-smokers as shown in [figure 1](#). There was significant difference on salivary MDA, micronucleus, pycnosis, karyorrhesis and karyolysis when analyzed with independent t-test ( $p < 0.05$ ).

Oneway ANOVA test result showed no significant change ( $p > 0.05$ ) on salivary MDA, micronucleus, pycnosis, karyorrhesis and karyolysis between smoker and non smoker group exposed to intraoral radiographs. There was no significant change in 10±2 days after P1 and P2 as shown on [table 1](#).

**Table 1** Comparison mean of micronucleus, pycnosis, karyorrhesis, karyolysis and salivary MDA levels before and after exposure to intraoral radiographs between non-smokers and smokers

Group	Genotoxic and Cytotoxic Indicators	Non Smokers		Smokers	
		mean±SD	p	mean±SD	p
MDA	P0	3.72±0.73		6.44±1.69	
	P1	3.81±0.73	0.902	6.55±1.69	0.973
	P2	3.92±0.72		6.69±1.71	
Mikronukleus	P0	2.4±1.14		18.4±5.5	
	P1	3.2±1.3	0.363	19.4±5.98	0.958
	P2	3.4±0.89		19.2±5.63	
Piknosis	P0	17.2±6.38		18.6±3.05	
	P1	18.4±7.16	0.790	20.8±2.28	0.196
	P2	20.4±8.41		21.2±1.09	
Karioreksis	P0	20.00±8.26		39.4±8.47	
	P1	32.4±9.34	0.873	41.4±8.29	0.917
	P2	32.8±7.59		41.4±8.29	
Kariolisis	P0	17.6±4.83		18.8±2.48	
	P1	19.2±3.11	0.782	21±2.83	0.287
	P2	19±3.53		21.6±3.05	

Analyzed with oneway ANOVA;  $p < 0.05$  = statistically significant



**Figure 1** Differences in micronucleus, pycnosis, karyorexis, karyolysis and salivary MDA between non-smokers and smokers before exposed to intraoral radiography

## Discussion

This study was carried out by taking exfoliated buccal epithelial cells and saliva as samples because they were considered easy in the process of taking and non-invasive procedure. Sampling for each patient was taken three times to find out the changes on micronucleus, pycnosis, karyorrhexis, karyolysis and salivary MDA levels as explained in methods.

That the number of micronucleus, pycnosis, karyorrhexis, karyolysis and salivary MDA levels in smokers was higher than non-smokers before exposure (P0). It can be seen from the comparison of mean micronucleus number and salivary MDA levels in smokers was higher compared to non-smokers caused by smoking habits that have occurred for years and exposed to genotoxic agents contained by cigarettes such as benzopirene and nicotine. Nicotine in cigarettes could be nitrosated and then transformed into nitrosamines which have the potential to become DNA adducts that initiated the formation of micronucleus.<sup>12,14</sup> Micronucleus were mainly derived from acentric chromosome fragments, acentric chromatid fragments or all chromosomes that fail to be included in the nuclei during telophase settlement in mitosis because these chromosome fragments or chromosomes did not adhere well to spindles during the separation process in anaphase. These unattached chromosomes or chromosome fragments were finally closed by nuclear membranes which were morphologically similar to the nucleus after conventional nuclear staining but in a smaller size.<sup>15</sup> This was in line with research conducted by Rahmah N et al.<sup>11</sup> which found that the number micronucleus in active smokers is higher than passive smokers.<sup>12</sup>

From the P0 comparison of smokers and non-smokers showed that salivary MDA in smokers was much higher than non-smokers. The higher

salivary MDA showed that the oxidative stress of oral mucosa cells in smokers was higher than non-smokers. The habit of smoking tobacco that was found around us could damage the oxidant balance and antioxidants in the body lead to free radicals. Free radicals derived from cigarettes or stimulated by smoking could lead to oxidative stress and initiated lipid damage to cell membranes.<sup>14</sup>

MDA as the end result of lipid peroxidation, was an active molecule that could adversely affect protein structure because it could diffuse easily around cells and tissues.<sup>16</sup> This is consistent with research conducted by Menicagli R et al.<sup>17</sup> stated that consumption 7 (seven) cigarettes a day will significantly increase salivary MDA levels compared to controls. Kurku H et al.<sup>18</sup> also conducted a study of the acute and chronic effects of smoking on saliva and found that smoking had an acute and chronic risk of causing cell oxidative stress and led to an increase in the amount of MDA in saliva.

In present study it was seen that there was no significant difference in number of micronucleus between P0, P1 and P2 in both smokers and non-smokers that could happen due to insufficient sensitivity of testing to detect changes after the exposure of intraoral radiographs with low doses although they exposed two times in a time. This was in line with a study conducted by Gang Li et al.<sup>18</sup> which found that there was no change in micronucleus number and cell death after exposure to radiation from low-dose radiodiagnostic.<sup>19</sup> And also supported by changes in salivary MDA that did not change significantly, seen from P0, P1 and P2 even though it had been repeatedly exposed. This could happen because there was a several days pause as interval between exposure to the first day (P1) and second day (P2) which could used by cells to repair themselves from damage before next exposure.

## Conclusion

There were no significant differences in genotoxic and cytotoxic indicators and salivary MDA levels, before and after the last exposure in smokers and non-smokers. It showed us that the use of intraoral radiographs was within safe limits despite repeated exposure with several days interval. However the repeated exposure effect more than 0,008 mSv (more than two times exposed) in a same time was remain unknown. As a practitioner in dentistry, radiographic prescribing should only be done if it is really needed.

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## Conflict of Interest

The authors report no conflict of interest.

## References

- White SC, Pharoah MJ. Oral radiology-E-Book: Principles and interpretation. Elsevier Health Sciences; 2014.
- Abbas AW, Zaidan TF, Al-barrak AYA. Assessment of serum and salivary malondialdehyde in patients with oral lichen planus. *J Bagh Coll Dent* 2014;26: 99-102.
- Amtha R, Kurniadi A. An overview of oral mucosa condition of shisha smoker. *J Dentomaxillofacial Sci* 2016;1: 67-70.
- Gowhar O. Comparison of exfoliative cytology of tongue and buccal mucosa among smokers and non-smokers using PAP stain and AgNOR counts. *Int J Contemp Med Res* 2017;4: 1587-1590.
- Kusuma A. alveolar bone loss, tooth loss. *J UNISSULA* 2011;1: 1-8.
- Khoubnasabjafari M, Ansarin K, Jouyban A. Salivary malondialdehyde as an oxidative stress biomarker in oral and systemic diseases. *J Dent Res Dent Clin Dent Prospects* 2016;10: 71-75.
- Cerqueira EMM, Gomes-filho IS, Trindade S, et al. Genetic damage in exfoliated cells from oral mucosa of individuals exposed to x-rays during panoramic dental radiographies. *Mutat Res* 2004;562: 111-117.
- Angelieri F, Yujra VQ, Tizuko C, et al. Do Dental x-rays induce genotoxicity and cytotoxicity in oral mucosa cells? a critical review. *Anticancer Res* 2017; 37: 5383-5388.
- Kesidi S, Maloth KN, Vinay K, et al. Genotoxic and cytotoxic biomonitoring in patients exposed to full mouth radiographs – a radiological and cytological study. *J Oral Maxillofac Radiol* 2017;5: 1-6.
- Smriti K, Muralidhar K, Ravindranath V. Science direct role of salivary malondialdehyde in assessment of oxidative stress among diabetics. *J Oral Biol Craniofacial Res* 2015;6: 41-44.
- Panganiban RM, Snow AL, Day RM. Mechanisms of radiation toxicity in transformed and. *Int J Mol Sci* 2013;14: 15931-15958.
- Rahmah N. Analisis Sitogenik Mukronukleus Mukosa Bukal pada Perokok Aktif dan Pasif. *Dentino* 2016;1: 15-20.
- Holland N, Bolognesi C, Kirsch-volders M, et al. Mutation research/reviews in mutation research the micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage : The HUMN project perspective on current status and knowledge gaps. *Mutat Res* 2008;659: 93-108.
- Ozturk O, Meydan A, Healthcare F. Effects of smoking on oral cavity. *J Exp Clin Med* 2017;34: 3-7.
- Fenech M, Natarajan AT. Molecular mechanisms of micronucleus, nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. *Mutagenesis* 2011;26: 125-132.
- Demirtaş M, Şenel Ü, Yüksel S, et al. A comparison of the generation of free radicals in saliva of active and passive smokers. *Turkish J Med Sci* 2014;44: 208-211.
- Menicagli R, Duca MRP. Development research monitoring of oxidative stress induced by cigarette smoke by analysis salivary malondialdehyde with the use of dipsticks. *Int J Dev Res* 2016;6: 7546-7551.
- Kurku H, Kacmaz M, Kisa U, et al. Acute and chronic impact of smoking on salivary and serum total antioxidant. *J Pak Med Assoc* 2015;65: 164-169.
- Li G, Yang P, Hao S, et al. Buccal mucosa cell damage in individuals following dental x-ray examinations. *Sci Rep.* 2018;1: 1-7.



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