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Assessment of Cytological Changes of Oral Mucosal in Smokers

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ABSTRACT

Tobacco use, both smoking, and chewing, is a risk factor for the development of oral mucosa changes. Tobacco in cigarettes causes an imbalance between antioxidant enzymes in metabolizing and detoxifying carcinogens in tobacco, which may cause changes in the oral epithelium that leads to lesions and dysplasia. This study aimed to assess and compare the cytological changes in the oral mucosal of smokers and non-smokers. Assessment of smokers based on the type of cigarette smoking, the degree of smoking, and the duration of smoking. The design study was a case-control study, as was a non-probability sampling method with consecutive sampling obtained from primary sources, including a questionnaire and an examination of epithelial cells under a microscope. The findings revealed that 14 smokers (38.9%) had changes in oral epithelial cells. The Fisher Exact Test on smoking habits and changes in oral epithelial cells based on the type of cigarette smoked ($p=0.001$), smoking degree ($p=0.000$), and duration of smoking ($p=0.003$) indicated a relationship between smoking-influenced cytological changes in the oral mucosal.

1. Introduction

Smoking is an activity that is often encountered in the community with the practice of burning tobacco, in which cigarette smoke is inhaled directly and absorbed into the bloodstream. The prevalence of smoking in the population aged over 10 years in Indonesia in 2018 was 28.8%. The prevalence of tobacco consumption in North Sumatra at the age of more than 15 years is 55.2%, with the type of cigarette smoked being kretek cigarettes. Meanwhile, in Medan City, the prevalence of active smokers is 18.16%, with the most active smoking age being 30-35 years old. Tobacco use in the form of smoking is a risk factor for the formation of lesions in the oral mucosa. Exposure to tobacco in cigarettes results in an imbalance between antioxidant enzymes in metabolizing and detoxifying carcinogens in tobacco, which may cause

changes in the oral epithelium that will develop into lesions and a state of dysplasia.¹⁻³

Based on the number of cigarettes consumed, smokers can be divided into three types, namely light smokers, moderate smokers, and heavy smokers. Someone categorized as a light smoker is one who consumes <200 cigarettes/year, a moderate smoker 200-599 cigarettes/year, and a heavy smoker who consumes 600 cigarettes/year. The number of cigarettes consumed by a person is directly proportional to the health of his mouth. Based on other studies, it was reported that there is a relationship between smoking and tobacco with the incidence of oral mucosal lesions. Another study found that the incidence of smoker's melanosis was most commonly found in subjects who had smoked for > 10 years. A study found that the risk of oral cancer in

smokers with kretek cigarettes and smoking for more than 10 years has a threefold risk factor compared to non-smokers. Another study found that there were dysplastic epithelial changes in smokers. The more cigarettes are consumed, the health status of the periodontal tissue will also worsen. It can be concluded that cigarettes have a significant relationship with cell changes in the oral cavity.⁴⁻⁶ This study aimed to assess and compare the cytological changes in the oral mucosal of smokers and non-smokers. Assessment of smokers based on the type of cigarette smoking, degree of smoking, and duration of smoking.

2. Methods

The study was an observational analytic with a case-control study to compare the cytological changes in the oral mucosa of smokers and non-smokers. The study was conducted in the anatomical pathology laboratory, Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara. Ethical clearance was obtained from the Faculty of Medicine, Universitas Muhammadiyah Sumatera Utara, before this study's commencement (Ethical clearance number: 611/KEPK/FKUMSU/2021). Sampling in this study used a non-probability sampling method with the type of consecutive sampling. The samples were smokers in Kelurahan Karang Berombak Medan who met the inclusion and exclusion criteria during the study period. The data taken in this study was primary data using a questionnaire including the history of duration of smoking, the types of cigarettes smoked, and the number of cigarettes smoked per day.

The specimens were taken by scraping (swabs) on the buccal part using a wooden spatula 3 times, scraping the same part with moderate pressure. Then it was applied to the object glass, and then the preparation was put into a specimen container which was filled with a fixation solution of 96% alcohol for 3-5 minutes and dried. The sample was dripped with eosin solution evenly on the object glass, then let stand

for 2-3 minutes, after which it was rinsed with running water and dried. The sample was dripped with a small amount of methylene blue solution and allowed to stand for 2-3 minutes, then rinsed with running water and dried. The preparation was covered with a covered glass and observed with a digital microscope for epithelial changes. Cytopathological features with cell changes can be seen under a microscope in the form of the presence of several pyknotic cell views per 10 small fields of view with 10x magnification. The results were analyzed using the Fisher exact test with a statistically significant difference if the value ($p < 0.05$).

3. Results and Discussion

The subjects of this study were 36 smokers and 36 non-smokers. The characteristics of the research subjects are shown in Table 1. The highest percentage of the group of smokers is aged 36-40 years, namely as many as 14 subjects (38.9%), and the lowest percentage of the group of smokers is aged 41-45 years, with as many as 6 subjects (16.7%). The highest percentage of the non-smoker group was aged 30-35 years, as many as 13 subjects (36.1%), and the lowest percentage in the non-smoker group was aged 46-50 years (16.7%), as many as 6 subjects (16.7%). Table 2 shows the distribution of cigarette types based on basic ingredients. The most used type of cigarette was non-filtered kretek cigarettes, with as many as 15 subjects (41.7%). Table 3 shows the distribution of smokers by degree of smoking according to the Brinkman Index. The category moderate smokers reached the highest frequency, namely as many as 14 subjects (38.9%), followed by mild smokers, as many as 12 subjects (33.3%), and severe smokers, as many as 10 subjects (27.8%). Table 4 shows the distribution of smokers by duration of smoking. The highest percentage of the duration of smoking is more than 10 years, as many as 26 subjects (72.2%), followed by the percentage of the duration of smoking less than 10 years, as many as 10 subjects (27.8%).

Table 1. Characteristics of the research subjects.

Age (years)	Smoker		Non-smoker	
	n	%	n	%
30 – 35	9	25	13	36.1
36 – 40	14	38.9	8	22.2
41 – 45	6	16.7	9	25
46 – 50	7	19.4	6	16.7
Total	36	100	36	100

Table 2. Distribution of cigarette types based on basic ingredients.

Cigarette type	n	%
White cigarette filter	12	33.3
Filter cigarettes	9	25
Non-filter kretek cigarettes	15	41.7
Total	36	100

Table 3. Distribution of smokers by degree of smoking according to the Brinkman index.

Smoker degree	n	%
Mild (< 200 cigarettes /year)	12	33.3
Moderate (200-599 cigarettes /year)	14	38.9
Severe (≥600 cigarettes /year)	10	27.8
Total	36	100

Table 4. Distribution of smokers by duration of smoking.

Duration of smoking	n	%
10 years	10	27.8
>10 years	26	72.2

Cytological changes in the oral mucosa in the form of pyknotic features, shown seen in Table 5 and Figure 2, were found in 14 smokers (38.9%). Figure 1 shows

normal squamous epithelial cells of oral mucosa in non-smokers.

Table 5. Prevalence of oral mucosa epithelial cell changes.

Oral mucosa epithelial cell changes	Smoker		Non-smoker	
	n	%	n	%
Yes	14	38.9	0	0
No	22	61.1	36	100
Total	36	100	36	100

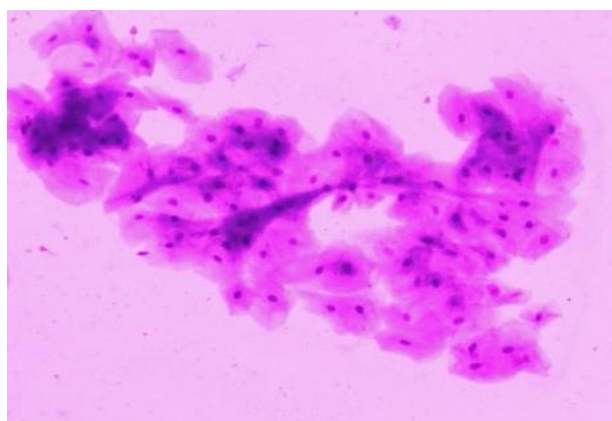


Figure 1. Oral mucosal epithelial cells in non-smokers (10x).

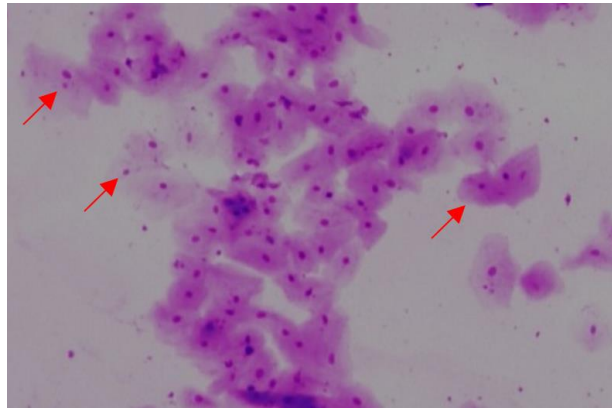


Figure 2. Oral mucosal epithelial cells in smokers show mild pyknotic (red arrow, 10x).

The relationship between cigarette types and cytological changes in oral mucosa epithelial cells in smokers was analyzed and is presented in Table 6.

Based on Fisher's exact test, it was found that there was a significant relationship between cigarette types and cytological changes in oral mucosa epithelial cells.

Table 6. The relationship between cigarette types and cytological changes in oral mucosa epithelial cells.

Cigarette type	Oral mucosa epithelial cells change				Total		p-value
	No		Yes		n	%	
	n	%	n	%			
White cigarette filter	11	91.7	1	8.3	12	100	0.001
Filter cigarettes	7	77.8	2	22.2	9	100	
Non-filter clove cigarettes	4	26.7	11	73.3	15	100	
Total	22	65.4	14	34.6	36	100	

The relationship between smoking degrees according to Brinkman index and cytological changes in oral mucosa epithelial cells in smokers was analyzed and is presented in Table 7. Based on

Fisher's exact test, it was found that there was a significant relationship between smoking degrees according to Brinkman index and cytological changes in oral mucosa epithelial cells.

Table 7. The relationship between smoking degrees according to Brinkman Index and cytological changes in oral mucosa epithelial cells.

Smoking degree	Oral mucosa epithelial cells change				Total		p-value
	No		Yes		n	%	
	n	%	n	%			
Mild (<200 cigarettes/year)	12	100	0	0	12	100	0.000
Moderate (200-599 cigarettes /year)	9	64.3	5	35.7	14	100	
Severe (≥600 cigarettes/year)	1	10	9	90	10	100	
Total	22	58.1	14	41.9	36	100	

The relationship between the duration of smoking and cytological changes in oral mucosa epithelial cells in smokers was analyzed and presented in Table 8. Based on Fisher's Exact Test, it was found that there

was a significant relationship between the duration of smoking and cytological changes in oral mucosa epithelial cells.

Table 8. The relationship between the duration of smoking and cytological changes in oral mucosa epithelial cells.

Duration of smoking	Oral mucosa epithelial cells change				Total		p-value
	No		Yes		n	%	
	n	%	n	%			
10 years	10	100	0	0	10	100	0.003
>10 years	12	46.2	14	53.8	26	100	
Total	22	73.1	14	26.9	36	100	

The relationship between smokers and non-smokers against cytological changes in oral mucosa epithelial cells was analyzed and is presented in Table 9. Based on Fisher's exact test, it was found that there

was a significant relationship between smokers and non-smokers against cytological changes in oral mucosa epithelial cells.

Table 9. The relationship between smokers and non-smokers against cytological changes in oral mucosa epithelial cells.

Group	Oral mucosa epithelial cells change				Total		p-value
	No		Yes		n	%	
	n	%	n	%			
Smoker	22	61.1	14	38.9	36	100	0.000
Non-smoker	36	100	0	0	36	100	
Total	58	80.5	14	19.5	72	100	

The types of cigarettes that are of interest to respondents in this study are non-filter kretek cigarettes, as many as 15 subjects (41.7%). It is because kretek cigarettes are more commonly consumed in Indonesia because these cigarettes contain a mixture of tobacco with cloves and clove oil which has a distinctive aroma. In addition, the difference between kretek cigarettes and white cigarettes is the eugenol content in kretek cigarettes which can cause psychotropic effects and local anesthetics and are also toxic, which can increase the risk of further disease. In addition, kretek cigarettes contain more tar, nicotine, and carbon monoxide, so smokers with kretek cigarettes are more at risk of developing epithelial cell changes in the oral cavity than those smoking white cigarettes. The degree of smoking, it was found that moderate degrees were the most respondents in this study, namely as many as 14 subjects (38.9%). The results of this study are in line with another study which stated that the highest degree of smoking was moderate, with a total of 45 respondents (63.3%). In another study, it was stated

that the highest degree of smoking was moderate smoking, which was 31 subjects (47.7%). It is because the large number of cigarettes smoked per day is influenced by the nicotine contained in cigarettes which causes a dependence effect on smokers, thus making smokers continue to increase the number of cigarettes smoked. Subjects who have the habit of smoking the most time are more than 10 years as many as 31 subjects (86.1%), followed by smoking less than 10 years as many as 5 subjects (13.1%). The results of this study are in line with another study, and it was found that most of the respondents in this study were more than 10 years old, with a total of 24 respondents (33.8%). In another study, it was stated that there were 37 respondents (48.7%) who had smoked for more than 10 years. There is a substance in cigarettes that can cause dependence on smokers. One of the substances contained in cigarettes is nicotine. This substance has an addictive effect, so subjects who smoke become dependent on these cigarettes. This is what makes some subjects smoke for decades.⁷⁻¹²

During the research, it was found that 14 smokers (38.9%) experienced changes in the epithelial cells in the oral cavity. This is in accordance with a study, and it was found that 30 smokers (25%) had leukoplakia lesions in the oral cavity with mild dysplasia. Another study stated that of 39 subjects who smoked, it was found that 12 smokers (30.8%) had leukoplakia lesions in the oral cavity. This is because smoking has an important role in the development of dysplasia in oral leukoplakia. It was found that smokers had a 9 times greater risk of developing oral dysplasia than non-smokers. The changes in oral epithelial cells occur more in respondents who smoked non-filtered kretek cigarettes, namely 11 subjects (73.3%). This is in accordance with another study, which stated that of a total of 2970 respondents who smoked non-filtered kretek cigarettes, 136 respondents (11%) of them experienced changes in epithelial cells in the oral cavity. The results of this study are also supported by another study, which states that the incidence of leukoplakia is more commonly found in the tobacco smoker group, with as many as 33 respondents (61.2%). Changes in the epithelial cells of the oral cavity are caused by the effects of tobacco on cigarettes, which will later develop into precancerous cells. This is because kretek cigarettes have a mixture of tobacco and cloves, which can increase the temperature of the cigarette when it is burned, thereby increasing carbon monoxide and nicotine gases, which provide higher toxicity to the oral mucosa, thereby increasing the risk of changes in epithelial cells in the oral cavity.¹³⁻¹⁸

The number of respondents who experienced changes in oral epithelial cells was mostly found in heavy smokers, namely, 9 respondents (90%). This is in accordance with the research, which states that there are changes in oral epithelial cells in heavy smokers, with as many as 13 respondents (16.25%). The results of this study are also supported by research by another study, which states that severe smokers have four times increased risk for oral cancer. This is caused by the large amount of tobacco smoke-exposed to the oral mucosa in smokers, causing

irritation that may lead to a lesion in the oral cavity. The most changes in oral epithelial cells occurred in respondents with a smoking period of more than 10 years, namely as many as 14 respondents (53.8%). This is in accordance with the other research, which states that of 65 subjects who smoked for more than 10 years, as many as 61 subjects (38.1%) experienced changes in oral epithelial cells. Another study, which stated that there were 4 respondents who smoked for more than 10 years, had precancerous lesions in the oral cavity. Smoking produces hot smoke that can irritate the oral mucosal tissue. Exposure to cigarette irritants that lasts a long time and continuously will cause injury to the oral mucosal tissue, and the oral mucosa will become thicker and appear as white patches.¹⁹⁻²²

Our findings showed no changes in oral epithelial cells in subjects, namely as many as 22 subjects (61.1%), and changes in oral epithelial cells were as many as 14 subjects (38.1%) and from 36 subjects who were not smokers did not the occurrence of changes in oral epithelial cells as many as 36 subjects (100%) and changes in oral epithelial cells as many as 0 subjects (0%). The results of this study are in accordance with research, which states that smoking has a significant effect on the occurrence of dysplasia compared to non-smokers. This study is also in line with research, which stated that there were 56 respondents smokers who experienced epithelial cell changes in the form of dysplasia in the oral cavity. Smokers cause the effect of genotoxic exposure directly on the oral mucosa both when smoking and when inhaling cigarette smoke. As a result, cells in the buccal mucosa can metabolize a carcinogenic agent into a reactive product. Therefore, DNA damage experienced in smokers will lead to the development of clinical symptoms, namely the occurrence of premalignant and malignant lesions on the oral mucosa.²³⁻²⁸

4. Conclusion

There were differences in the cytology of oral epithelial cells in smokers and non-smokers based on the type of cigarette smoked, the degree of smoking,

and the duration of smoking, and there were relationships between smoking-influenced cytological changes in the oral mucosal.

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