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The nuclear cytoplasmic ratio analysis of smoker's oral mucous epithelium cells

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Abstract

Background: Smoking has side effects for the body that one of them may manifest as malignancy in the oral cavity. One of the main contributing factors that trigger malignancy is carcinogenic substances. These carcinogens can cause malignancy that may be detected through the changes of cells in form of an increase of diameter of the nucleus and the nuclear cytoplasm ratio (N/C-R). Objective: To evaluate the N/C-R value of the oral mucous epithelium of academic community smokers at Universitas Trisakti. Methods: This research is an analytical observational study through exfoliate cytology procedure used scrapping technique and Papanicolaou staining to evaluate the nuclear, cytoplasmic diameters, and N/C-R value of oral mucous epithelium cells. This study involved 60 subjects divided into two groups: 30 smokers and 30 non-smokers. Cells evaluation at three fields of each cytology slide was done by measuring the diameter of nucleus, cytoplasm and N/C-R of each cell that is not undergoing micro nucleus neither karyorrhexis or karyopyknosis. Results: There was no significant difference in the N/C-R (p=0,156>0,05) between the smoker and non-smoker group even the N/C-R value of the smoker group is higher (0,19+0,03908>0,18+0,02618). Conclusion: It was observed that smokers have a higher N/C-R value compared to those of non-smokers with no significant difference and still in the range of normal N/C-R value.

Keywords: Smoking; Carcinogenic; Oral Mucosa Epithelium Cells; N/C-R

1. Introduction

Smoking is the third leading cause of death in Indonesia. The prevalence of young smokers (10-18 years old) continues to rise from 7.2% at the year of 2013 to 9.1% at 2018.[1] In Indonesia, smoking becomes a population habit that give bad impact for the environment subjects both from the aspects of health and psychology. At 2019, the number of tobacco users from the age of 13 to 15 years old was 19.2% with 35.6% of them are boys and 3.5% are girls.[2] World Health Organization (WHO) recorded that as much as 225.700 death people due to yearly smoker's habit. Another study revealed that during these 5 years (2013-2018), adult smoking prevalence has not been decrease yet, however the young population (10-19 years) prevalence showed increased as much as 2% (from 7.2% to 9.1%).[3] The *Global Adult Tobacco Survey* Indonesia (2021) revealed that electric smokers increase at adult as much as 516.377 person at the year of 2011 to more than 6 million at 2021. Now a days, the young age from senior high school to university student already started having smoking habit with the increase amount.[4]

Carcinogenic effect of smoking habit induces clinical changes in the oral mucous, such as *mucous hyperkeratosis* in form of *leukoplakia* to *erythroleukoplakia* at the buccal mucous, lip, tongue and floor of mouth. Carcinogenic compounds in tobacco, including *nitrosamine* and *nitrosonornicotine* trigger cellular alterations, such as nuclear enlargement and DNA mutations, increasing the risk of malignancy especially in oral cavity.[5,6,7.]

Nitrosamines and *N-Nitrosonornicotine* are carcinogenic substances in tobacco that have an important role in carcinogen process of cellular changes started from the apoptosis to molecular reactivity including the DNA that finally become

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gene mutation or malignancy. The accumulation of *nitrosamines* causes the protein synthesis activity in form of the enlargement of cell nuclear as responds of the cellular destruction. The enlargement of nuclei is the first sign of carcinogen process. *Nitrosamine* also increase the amount of *Reactive oxygen species* (ROS), that promote oxidative stress respond.[8]

Smoker habit especially its *nitrosamine* content of tobacco increases malignancy risk due to the damage of DNA within oral mucous epithelium with the process started from the chronic irritation followed by chronic inflammation that influenced the cellular genetic (DNA) stability. The damage of oral epithelium cell DNA promotes the mutation of tumor suppressor and proto oncogenes that increase the risk of lesion transformation into cancer such as *erythroplakia* into *squamous cells carcinoma*.[9] From the aspect of science and public health, the study of smoking impact is very important in proper to evaluate the early sign of malignancy as its effect on oral epithelium cells. However, there is not enough research about the changes of cells used as indicator of malignancy such as the N/C-R value of smokers oral epithelium cells therefore this study was done to evaluate the oral mucous smokers of Civitas Academica Universitas Trisakti.

2. Materials and Methods

This observational analytic study aim is to analysis the smokers' influence toward nuclear cytoplasmic ratio (N/C-R) value of oral epithelium cell. This study that was done at Faculty of Dentistry Universitas Trisakti on October, 2024 already got ethical approval from Ethical Medical Research Commission of Dental Faculty Universitas Trisakti with the number: No.850/S1/KEPK/FKG/7/2024.

The research subjects are Civitas Academica at Universitas Trisakti. The sample size decided used G* power application and *Consecutive technique sampling* from subjects that fulfilled the inclusive and exclusive criteria. The inclusive criteria are: 1) smoking man; 2) conventional and electric smokers; 3) minimal last 5 years of smoking history; 4) minimal use 10 stick each day. The exclusion criteria are: 1) has systemic disease history; 2) has habit cheek biting ; 3) use removable denture.

The total research subjects are 60 consisted of 30 smoking and 30 nonsmoking with the characteristic as shown on Table 1.

Table 1 Research subject characteristic

Characteristic	**Smoking**		**Non smoking**	
	Total	**Percentage**	**Total**	**Percentage**
Age (year)				
Mean		28,27		19,84
Modus		21		20
Youngest		19		17
Eldest		64		23
Profesion				
Modus		21		20
Youngest		19		17
Eldest		64		23
Modus		21		20
Youngest		19		17
Eldest		64		23
Modus		21		20
Type of smoking				
Conventional	18	60%	- -	-

Electric	2	7%	-	-
Combined				
Conventional				
White cigarrette	18	60%	-	-
Kretek	10	33%	-	-
Combined	0	0%	-	-
Electric type				
Pod	9	30%	-	-
Mod	2	7%	-	-
Iqos	1	3%	-	-

The clinical material sample of research subjects was collected by scrapping technique at Campus A and Campus B Universitas Trisakti. The cytology slide formation of those clinical materials used Papanicolaou staining and the microscopic evaluation at *Oral Pathology for Diagnostic, Collaboration Research and Education Laboratory (OPaDCORE*) of Faculty of Dentistry, Universitas Trisakti.

The microscopic evaluation was done from representative three areas selected from each slide used the magnification of 40x10. The nuclear, cytoplasm size and the N/C-R value of each cell that has not shown the features of micro-nucleus, karyorrhexis, or crowded was measured used Image J application (Figure 1 and 2).

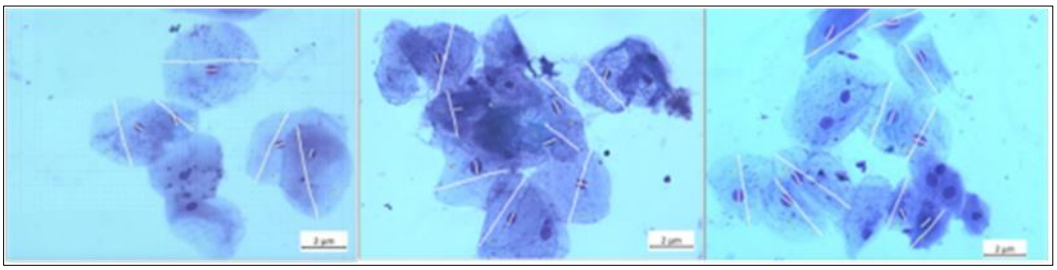


Figure 1 Nuclear and cytoplasm of smoking oral mucous epithelium cells size measurement (Magnification 40X10 and ratio 2 µm)

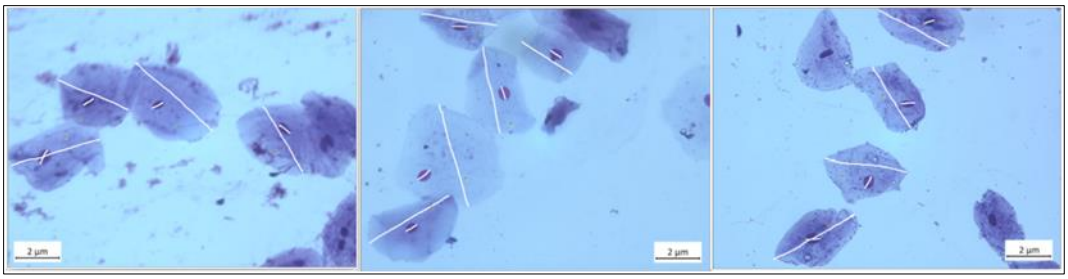


Figure 2 Nuclear and cytoplasm of non-smoking oral mucous epithelium cells size measurement (Magnification 40X10 and ratio 2 µm)

The data was analyzed statistically by Kolmogorov-Smirnov test followed by the independent T-test for the homogeneous and Mann Whitney for the non-homogeneous one.

3. Result and Discussion

The average size of nuclear, cytoplasm, and N/C-R cells value of smoking and non smoking group are shown on Table 2, 3, 4.

Table 2 The average of nuclear size

	Group	N	Mean	Std. Deviation	Std. error mean
Average of nuclear size	Smoking	30	0.7060	0.13650	0.02492
	Non-smoking	30	0.6173	.08473	0.01547

Table 3 The average of cytoplasm size

	Group	N	Mean	Std. Deviation	Std. error mean
Average of cytoplasm size	Smoking	30	3.7760	0.97844	0.17864
	Non-smoking	30	3.5157	0.49687	0.09072

Table 4 The average of N/C-R value

	Group	N	Mean	Std. Deviation	Std. error mean
Average of N/C-R value	Smoking	30	0.1937	0.03908	0.00714
	Non-smoking	30	0.1790	0.02618	0.00478

On Table 2, the average of nuclear size on smoking group (0.7060$\pm$0.1365) higher than those of nonsmoking (0.6173$\pm$0.08473) as well as their N/C-R value (0.1937$\pm$0.03908 > 0.1790$\pm$0.2618) which showed on Table 4.

The Kolmogorov-Smirnov test used to compare between two groups consisted of 60 respondents (Table 5, 6, and 7).

Table 5 *Kolmogorov-Smirnov* test of nuclear size data

	Group	statistic	df	Sig.
Average of nuclear size	Smoking	0.171	30	0.026
	Non-smoking	0.167	30	0.032

Table 6 *Kolmogorov-Smirnov* test of cytoplasm size data

	Group	statistic	df	Sig.
Average of cytoplasm size	Smoking	0.156	30	0.060
	Non-smoking	0.115	30	0.200

Table 7 *Kolmogorov-Smirnov* test of N/V-R value data

	Group	statistic	df	Sig.
Average of N/C-R value	Smoking	0.137	30	0.154
	Non-smoking	0.168	30	0.031

Table 5 showed that the nuclear size distribution of smoking and nonsmoking group was not normal (0.026<0.05) and (0.032<0.05) therefore the statistical analysis will be continued with non-parametric test of *Mann-Whitney* that showed on Table 8.

On Table 6 the cytoplasm size distribution of smoking and nonsmoking group was normal which are (0.060>0.05) and (0.200>0.05).

On Table 7, the N/C-R value of smoking group was distributed normal (0.154>0.05) which different with those of nonsmoking group (0.031<0.05) therefore the analysis will be continued with non-parametric test of *Mann-Whitney* as shown on Table 9.

Table 8 *Mann-Whitney* test of nuclear size

	Nuclear size
Mann-Whitney U	278.500
Wilcoxon W	743.500
Z	-2.539
Asymp. Sig. (2-tailed)	0,011

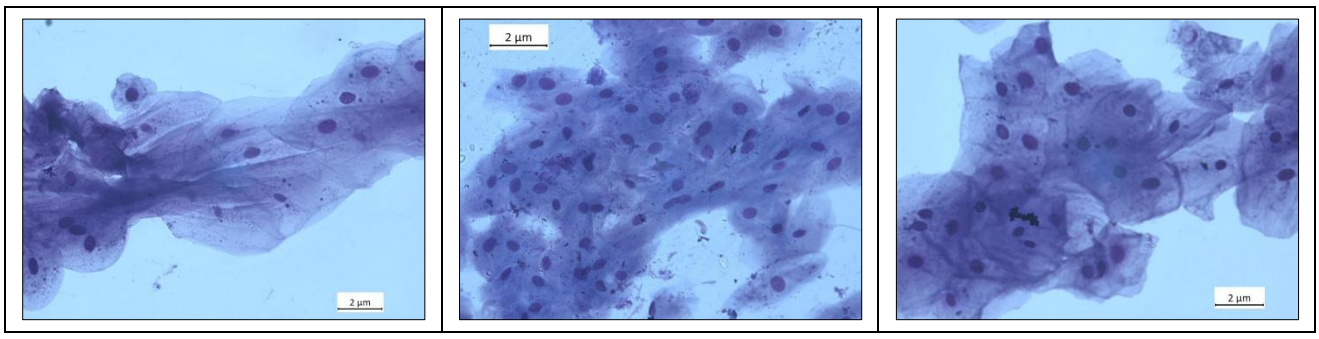
On Table 8, it was shown that there was significant difference of the average of nuclear size between smoking and non smoking group (p=0.011<0.05).

Table 9 *Mann- Whitney* test of N/C-R value

	N/C-R
Mann-Whitney U	354.500
Wilcoxon W	819.500
Z	-1.420
Asymp. Sig. (2-tailed)	0,156

Table 9 showed that there were no significant differences of the N/C-R value between smoking and nonsmoking group (0.156>0.05).

Table 10 Cells with the increase of N/C-R normal value



Only three (3) of 30 smoking groups subjects showed the increase of N/C-R value that was higher than normal cell which were (0.26>0.25; 0.26>0.25 and 0.28>0.25) as shown on Table 10.

Table 11 *Independent T-test* of cytoplasm size

	Subject	**One sided**	**Two sided**	**Mean difference**
Average of cytoplasm size	**Equal variances assumed**	0.099	0.199	0.26033
	Equal variances not assumed	0.100	0.201	0.26033

Independent T-test showed that is not significant differences between cytoplasm size of smoking and nonsmoking group (p=0.201>0.05) as shown on Table 11.

Cigarettes contain tobacco consisted of toxic and carcinogenic substances such as nicotine, tar, arsenic, formalin and carbon monoxide that cause the changes in structure, coordination toward malignancy of human cells, tissue, and organ.[8] Vape or electric smoking is often suspected more safety however the toxic effect toward the human body as dangerous as conventional type of smoking. Vape also give dangerous side effect due to the most of its contents is nicotine.[10] Smoking habit give eight-time higher risk to have carcinogenic effect on human body malignancy included oral cavity that in form of squamous cell carcinoma.[11] Carcinogenic effect of smoking on oral mucous epithelium cells can be evaluated microscopically through nuclear and cytoplasm cellular changes that show hyperchromatic nuclei and increase of nuclear size caused by the increase of DNA composition. The increase of nuclear size resulted in the increase of N/C-R value. These changes showed the over transcription activity and genetic replication. The nuclear cell also show variation in size and morphology become polymorphic reflected the unstable genetic and loss of control in proliferation activity. These characteristic malignant changes of cells caused of the destruction of DNA and the loss of cell cycle mechanism regulation by carcinogen effect of cigarette smoke. Each time of smoking activity resulted in the million of compound included more than 60 carcinogenic substances. Carcinogen within cigarette smoke consisted of several chemical groups such as *polycyclic aromatic hydrocarbons* (PAH), *N-nitrosamine, aromatic amine*, *aldehyde*, *evaporated organic hydrocarbon* and *meta*l that is proven has an important role in human cancer formation related to smoking.[12]

Early cellular changes of malignancy able to be evaluated microscopically in form of the changes of nuclear and cytoplasm size or N/C-R value where there is the nuclear hyperchromatic and the increase of nucleus that not proportional to cytoplasm size toward the increase of N/C-R value.[13] The increase of N/C-R value marked by the abnormality as polymorphous of nuclear and its size followed by uncontrolled cells proliferation and mutation time.[14]

This study showed the average nuclear size of smoking group is larger (0,70±0,13650>0,62±0,08473) and also significant difference between smoking and nonsmoking group with p=0,011<0,05. The average value of N/C-R in smoking group is also larger (0,19±0,03908>0,18±0,02618) without significant differences with p=0,156>0,05. However the average of N/C-R value both in smoking and nonsmoking group did not show the increase of normal N/C-R value which were (0.19<0.25 and 0.18<0.25). There is just three subjects of smoking group that showed higher N/C-R normal value (0.26>0.25 ; 0,26>0.25 and 0,28>0.25). This significant difference of the average nuclear size with the larger size in smoking group found in this study probably due to carcinogen effect of nitrosamine and nitrosorcotine within tobacco.

The study of Aigbogun et al, showed significant decrease of cytoplasm and the increase of nuclear size and N/C-R in smoking subjects.[15] The study stated that the cytoplasm decrease caused by the decrease of salivary flow around cells while the increase of nuclear size by pathological changes as early transformation toward malignancy. According that study, the passive smoking subject has no significant differences due to having very mild smoking exposure. That study is proper to the previous study by Komal Khot, et al. about the impact of tobacco in oral health by using exfoliate cytology method to know about cellular morphological changes. This 36 smoking and 36 nonsmoking study concluded that the nuclear size and N/C-R value increase within the smoking group.[16]

The human resistance toward carcinogen influenced by the genetic of enzyme CYP450 that arrange the carcinogenic effect in human body. Human respond toward carcinogenic substances also influenced by BRCA1/2 as DNA repair gen that play a role in decreasing carcinogenic effect in human body.[15] The N/C-R value that is in normal range value and not in significant difference between smoking and nonsmoking group found in our study revealed that smoking subjects have good defence mechanism as human resistance against carcinogenic agents of tobacco even though there is still showed that the nuclear size of smoking group is higher than those of nonsmoking with significant difference. Another factor that influenced the subject resistance in our study is systemic disease as one of the subjects' exclusion criteria. Human with systemic disease has lower immune resistance that will be not maximum responding the carcinogen. The research of subjects with diabetes showed the higher risk to cancer due to lower immune respond against carcinogen substances.[17]

4. Conclusion

Smoking has carcinogenic effect through oral mucous epithelium cells changes that has been proven in this study by the larger nuclear size within smoking group compared to those of nonsmoking with significant difference. There was also shown from their higher N/C-R cells value even though still in the range of normal value.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

This study already has ethical approval from Ethical Medical Research Commission of Dental Faculty Universitas Trisakti with the number: No.850/S1/KEPK/FKG/7/2024. Informed consent was obtained from all individual participants included in this study.

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