Buccal swab, a minimally invasive method for the screening of oral cancer in active smokers

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Abstract. Smoking is the main risk factor for developing oral cancer. The previous study showed that there was a strong correlation between the length of smoking with the risk to develop oral cancer. Early detection of epithelial changes of oral mucosa will be a good prevention of the incidence of oral cancer among active smokers. This study evaluated the potential use of buccal swab for the screening of early signs of malignancy in active smokers. This study involved 80 participants including those who were smokers and non smokers. The buccal swab was conducted using sterile cytobrush. An epithelial smear was made from the buccal swab and stained with Papanicolaou's technique. An cytomorphometric analysis was conducted by comparing the ratio of nuclear cell to cytoplasmic diameter (ND/CD) between the two groups. The mean of ND observed in this study were 8.963µ for active smokers and 7.991µ for non smokers groups. While the mean of CD were 58.249µ and 63.473µ for active smoker and non-smoker respectively. The mean of ND/CD ratio were 0.156 for active smokers and 0.129 for non smokers groups. This study detected a significant difference on the ND/CD ratio among active smokers vs non smokers (p<0.0001; 95% CI = -0.040 – -0.014). In conclusion buccal swab could be a routine procedure to obtain sample for identification of changes in cells morphology to screen an early development of oral cancer.

1. Introduction
Oral cancer is cancer which occurs on mouth, tongue, and oropharynx. The incidence of oral cancer is quite high with 300,286 cases worldwide were reported in 2012. Compared to other regions, the incidence and mortality rate of oral cancers in South East Asia Region is the highest. In fact, 34.46% (103,464/300,286) of oral cancer cases worldwide are in South East Asia Region [1]. Among the South East Asia countries, Indonesia reported the highest incidence of oral cancer. A number of 5329 cases were reported in Indonesia in 2012 and this incidence is predicted to increase year by year [2]. Although these data showed a serious problem, there is a lack of awareness of health society toward oral cancer. A study in Dharmais National Cancer Hospital of Indonesia showed that most of the patients with oral cancer seek medical help in severe conditions, with the majority of patients were first diagnosed in stage 4; this condition explains the high mortality rate of oral cancer patients in Indonesia [3].

The efforts to prevent oral cancer incidence should include programs to educate the society about the risk factors of oral cancer and to detect early cancer development. There are several risk factors for oral cancer development including, smoking, alcohol consumption, and HPV infection [1,4]. It has
been shown that tobacco smoking is strongly correlated to oral cancer incidence. In India, 74% of oral cancer patients had history of long term tobacco used [4]. As part of detection oral cancer, biopsy followed by histopathology examination of a clinically pathologic lesion is recommended as an early detection of oral cancer [5]. However, this effort is quite late, considering that the process of cancer development has been started at a long time before the clinical lesion can be observed. This study aims to assess the potential of buccal swab as a minimally invasive method to collect a sample source for detection of early oral cancer development in active smokers.

2. Material And Methods
This study involved 80 male participants; 40 active smokers and 40 nonsmokers. The inclusive criteria for an active smoker are smoking over 10 years and consuming at least 10 cigarettes per day. Participants with diabetes mellitus, a history of radiotherapy and or chemotherapy were excluded from this study. The nonsmokers participants were those who were not smoking nor being passive smokers, not diabetic patient and not having a history of radiotherapy or chemotherapy. Research were conducted in District Surakarta, Central Java, Indonesia. Participants were selected by convenient sampling method; a person who meets the subject criteria are asked to be research participants. All participants were given written informed consent and the research protocol has been approved by Ethics Committee of Muwardi Hospital and Faculty of Medicine Universitas Sebelas Maret, Indonesia.

2.1. Buccal Swab
Buccal swab for a collection of oral epithelial samples was conducted using a sterile cytobrush. The participants were instructed to brush the teeth and do mouth washing thoroughly with water prior to epithelial swab. The epithelial swab was taken from buccal and tongue areas by stirring the cytobrush for 5 times on the areas. An epithelial smear was made from the buccal swab and then the preparation was stained with Papanicolaou’s technique for cytomorphometric measurement.

2.2. Cytomorphometry
The cytomorphometry was conducted by measuring the ratio of nuclear cell diameter (ND) vs cytoplasmic cell diameter (CD) on 40 cells per individual Papanicolaou’s smear. Measurement of ND and CD were made using Optilab Image Raster software® under 1000x microscopic magnification, as can be shown in figure 1. The difference in ratio of ND to CD was analyzed statistically using Independent t-test.

3. Result
Characteristics of participants are distributed according to age, length of smoking, amount of cigarette smoked, daily habit of oral hygiene, and alcohol consumption. These characteristics are shown in Table 1. The age of participant involved in this study range from 21 to 73 years. The average of age among active smokers and nonsmokers groups (43.05 vs 43.95 years) is not significantly different (p= 0.743). The data on oral hygiene habit which is categorized based on the frequency of tooth brushing also showed that there were no differences in oral hygiene habit between active smokers and nonsmokers groups (p= 0.817). However, there is a different number of participant consuming alcohol between the two groups. The number of participant who consumes alcohol is higher in active smokers group compared to nonsmokers group (14/40 vs 2/40).

3.1. Cytomorphometry
The mean of CD and ND of epithelial cells collected from active smokers are 58.249µ and 8.963µ respectively. On the other hand, the mean of CD and ND are 63.473µ and 7.991µ for epithelial cell collected from nonsmokers. On average epithelial cells collected from active smokers have smaller cells size with bigger nuclear size compared to those collected from nonsmokers. The means of ND/CD ratio are 0.156 and 0.129 for active smokers and nonsmokers respectively. The ratio is higher
for active smokers compared to nonsmokers. A significant difference of the ND/CD ratio was observed between the active smoker and non-smoker p<0.0001 (95% CI = -0.040 to -0.014) (Table.1).

Table 1. The Characteristic of Participants. Participants are Distributed According to Age, Frequency of Toothbrushing, Alcohol Consumption, Length of Smoking and The Number of Cigarette Consumption Per day. The Cytomorphometry Result is Also Shown.

| Participant | active smoker | Non-smoker |
|-------------|--------------|------------|
| Age (p=0.743) | | |
| Min | 25 | 21 |
| Max | 73 | 66 |
| Mean | 43.050 | 43.950 |
| STDev | 10.561 | 13.679 |
| Toothbrush frequency (p = 0.817) | | |
| 1x | 7 | 6 |
| 2x | 28 | 31 |
| 3x and more | 5 | 3 |
| Alcohol use | | |
| Frequently | 8 | 0 |
| Rarely | 6 | 2 |
| Never | 26 | 38 |
| Smoking years | | |
| Min | 10 |
| Max | 54 |
| Mean | 25.875 |
| Cigarette amount | | |
| Min | 12 |
| Max | 40 |
| Mean | 18 |
| Cytomorphometry (p < 0.001) | | |
| Mean CD | 58.249µ | 63.473µ |
| Mean ND | 8.963µ | 7.991µ |
| Mean ND/CD | 0.156 | 0.129 |

4. Discussion
Smoking has been widely considered as a risk factor for cancer [6]. The effects of smoking on oral epithelial mucous include the increase of micronuclei, changes in keratin pattern and dysplasia. Examination of epithelial morphology for investigating the effect of smoking on buccal epithelial cells showed the increase in the formation of DNA adduct in heavy smokers [7]. Another parameter to measure the changes in epithelial cells lining the oral mucosa is by cytomorphometric analysis. This measurement gives information on the changes in average cells size from buccal epithelial smear. A cytomorphometric study revealed that the decrease in cytoplasmic area and the increase in the nuclear
Figure 1. Measurement of Nuclear Diameter and Cytoplasmic Diameter on Epithelial Smear Stained with Papanicolaou’s Technique Under 1000x Microscopic Magnification

Area of epithelial cells are associated with the development of malignancy [8]. The increase of nuclear size is one of the histological signs of epithelial dysplasia. Cellular changes including the increase ratio of nuclear to cytoplasmic size can be an important finding in the detection of epithelial dysplasia prior to the clinical manifestation of early cancer lesion. Other cellular appearances of epithelial dysplasia are hyperchromatism and prominent nucleoli [1,8].

Using the sample collected from the buccal swab, this study shows a valuable information on the morphological finding of oral epithelial cells exposed to cigarette smoking. The cytomorphicetric measurement in this study shows that the mean of ND of epithelial cells is higher in active smokers compared to the mean in nonsmokers. On the other hand, the mean of CD is lower in active smokers compared to the mean of CD in nonsmokers. These data indicate that the average cell size of epithelial cells collected from active smokers is smaller than the average size of cells collected from nonsmokers. The bigger nuclear cells size in active smokers than the size in nonsmokers predicted the effect of smoking on epithelial cells morphology. The significant difference of ND/CD ratio between active smokers and nonsmokers in this study gives a stronger suggestion on the contribution of smoking to epithelial dysplasia. In conclusion, the result of this study shows that a simple procedure like buccal swab can be an easy way to screen early morphological changes on oral epithelial mucosa to prevent the development of oral cancer. Further study is required to establish this method for oral cancer screening.

5. References

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