Folic Acid Usual Doses Decrease the Buccal Micronucleus Frequency on Smokers

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Abstract

Cigarette contains toxic chemical compounds that trigger DNA instability. Initial genotoxic oral cavity characterized by the appearance of micronucleus (MN) in the buccal mucosa. Folate is needed in maintaining DNA stability. This study aimed to compare the effects of folic acid usual doses (400 mcg and 1,000 mcg) on the MN frequency of buccal mucosa in active smokers. It is a clinical trial conducted in November 2018 in the Laboratory of the Faculty of Medicine, Universitas Islam Bandung of 53 active smokers who divided into two treatment groups. Group A was administered by 400 mcg and group B 1,000 mcg folic acid supplementation within three weeks. The buccal mucosa smear stained with hematoxylin-eosin (HE) and observed through a light microscope with 100× and 400× magnification. Data were analyzed by the Wilcoxon test statistically. The results showed that there was a significant decrease (p=0.00) in MN frequency in folic acid supplementation for three weeks, namely group A=6.39±3.92 and group B=6.93±5.82 in pre-supplementation, and group A=3.80±2.66 and group B=3.31±2.71 post-supplementation of folic acid. Giving a dose of 400 mcg and 1,000 mcg for three weeks did not provide significant results (p=0.94) with Kruskal-Wallis test. In conclusion, administration of folic acid at usual dose give results to a decrease in the buccal mucosa MN frequency in active smokers.

Key words: Active smokers, folic acid, micronucleus

Asam Folat Dosis Lazim Menurunkan Frekuensi Mikronukleus Mukosa Bukal pada Perokok

Abstrak

Asap rokok mengandung senyawa kimia toksik yang memicu ketidakstabilan DNA. Deteksi genotoksik awal rongga mulut ditandai dengan kemunculan mikronukleus (MN) pada mukosa bukal. Folate diperlukan dalam menjaga kestabilan DNA. Penelitian ini bertujuan mengetahui efek asam folat dosis lazim (400 mcg dan 1,000 mcg) terhadap frekuensi MN mukosa bukal pada perokok aktif. Penelitian ini merupakan uji klinis yang dilakukan pada bulan November 2018 di Laboratorium Fakultas Kedokteran, Universitas Islam Bandung terhadap 53 perokok aktif yang dibagi ke dalam dua kelompok perlakuan. Kelompok A mendapatkan suplementasi asam folat 400 mcg dan kelompok B mendapatkan suplementasi asam folat 1,000 mcg selama tiga pekan. Apus mukosa bukal diwarnai dengan hematoxylin-eosin (HE) dan diamati melalui mikroskop cahaya dengan pembesaran 100× dan 400x. Data dianalisis dengan uji Wilcoxon secara statistik. Hasil penelitian menunjukkan bahwa terdapat penurunan frekuensi MN yang signifikan (p=0.00) pada pre-suplementasi asam folat dosis 400 mcg dan kelompok B=3,31±2,71 pada post-suplementasi, serta kelompok A=6,39±3,92 dan kelompok B=6,93±5,82 pascasuplementasi asam folat. Pemberian dosis 400 mcg dan 1,000 mcg selama tiga pekan tidak memberikan hasil yang bermaquina (p=0,94) berdasar atas Uji Kruskal-Wallis. Simpulan, pemberian asam folat dosis lazim memberikan hasil baik terhadap penurunan frekuensi MN mukosa bukal pada perokok aktif.

Kata kunci: Asam folat, mikronukleus, perokok aktif

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Introduction

Smoking is one of the unethical lifestyles associated with genetic damage. Tobacco contains toxic chemical compounds and free radicals which can trigger cell damage and induces mutations in the deoxyribonucleic acid (DNA) which will increase the risk of malignancy in the oral cavity, lungs, and other non-communicable diseases.1–3

Indonesia had the first largest number of smokers in the world in 2015; there were 76.2% of smokers who were aged 15 years and older. West Java contributes to the 4–6 times increase in the proportion of active smokers at 1% and passive smokers at 62.8%.3 A total of 10.9 million residents of the Bandung city became smokers by spending 12–29 cigarettes per day.4

The buccal mucosa (BM) is a covered flat epithelial layer that functions as a protection for the oral cavity against potential carcinogenic substances that can produce potential reactive products in the oral cavity. The cells in the BM layer used to detect the occurrence of initial genotoxins caused by carcinogenic inhalant substances such as cigarette smoke. Exfoliated buccal cells used as non-invasive indicators of genotoxic effects characterized by the appearance of MN in cells. The frequency of the micronucleus is proportional to the degree of exposure and the potential of carcinogenic substances.5 An examination of buccal MN cells can be done using HE staining through observation of a light microscope or specific DNA stainings such as acridine orange and Feulgen. This method can be used regularly as an initial screening in groups of individuals who are susceptible to exposure to carcinogenic substances.6,7 Based on the description above, the researcher was interested to see the effects of folic acid at usual dosage (400 mcg and 1,000 mcg) to the frequency of buccal mucosa MN in active smokers.

Methods

The 60 participants selected regarding the duration of smoking habits; minimum for one year. The participants should have no alcoholic consumption habits, systemic diseases, allergy to folic acid, undergoing radiotherapy, performing oral x-ray examination procedures in the last month, amalgam dental fillings, and chronic infection, and inflammation of the oral cavity. Fifty-three participants follow the entire three weeks of the research period.

The samples swabbed from the buccal mucosa using a buccal brush at pre-supplementation and post-supplementation group of 400 mcg and 1,000 mcg folic acid. The buccal smeared on the glass object and stained by HE. The results were observed on 100 cells and evaluated with 100× and 400× magnification using a light microscope.

The data analyzed using the Statistical Package for Social Sciences software (SPSS Inc., Chicago, IL, United States). Data presented as a mean and standard deviation. The differences between the variables were analyzed using the Wilcoxon and Kruskal-Wallis test. A p value of <0.05 was considered significant. This research had approved by the Health Research Ethics Committee of Faculty of Medicine Universitas Islam Bandung with the ethical clearance number: 379/Komite Etik.FK/X/2018.

Results

The MN in the buccal mucosa found in varying amounts between participants. Micronucleus
observations are carried out through a light microscope with 100× and 400× magnification with calculations performed on 100 cells in each buccal mucosa smear. The size of MN is $\frac{1}{3}-\frac{1}{6}$ times than the central nucleus, but they have similar round shape and density. The micronucleus images found in this study presented in Figure 1 and 2 below.

The comparison of MN frequency between the participant who were provided 400 mcg and 1,000 mcg folic acid as pre-supplementation and post-supplementation are shown on Table.

In the group of participants who consumed 400 mcg (group A) and 1,000 mcg (group B) the frequency of the post-supplementation micronucleus was lower (group A=3.80±2.66; group B=3.31±2.71) than the pre-supplementation MN (group A=6.39±3.92; group B=6.93±5.82) respectively. There was a significant difference between the frequency of the MN pre-supplementation and post-supplementation of folic acid, both in the group provided with folic acid at 400 mcg and 1,000 mcg. Therefore the implementation of 400 mcg of folic acid was able to significantly reduce the buccal mucosa MN frequency of active smokers, while the dosage of the 400 mcg and 1,000 mcg did not give any significant effect.

Discussion

Tobacco use habits in various forms are associated with an increased risk of oral cancer.11 Cigarettes

![Figure 1 (A) Normal Cell, (B) Micronucleus in Buccal Mucosa](image1)

![Figure 2 Micronucleus in Buccal Mucosa Cell (arrow head)](image2)

Table  Comparison of MN Frequency between Group

| Supplementation      | MN Frequency Group A | MN Frequency Group B | p Value |
|----------------------|----------------------|----------------------|---------|
| Pre-supplementation  | 6.39±3.92            | 6.93±5.82            | 0.00*   |
| Post-supplementation | 3.80±2.66            | 3.31±2.17            | 0.00*   |
| Δ                    | 2.59±1.26            | 3.32±3.65            | 0.94**  |

Group A: participants who were provided with 400 mcg folic acids; Group B: participants who were provided with 1000 mcg folic acids; *Wilcoxon test, significance if p<0.05; **Kruskal-Wallis test, significance if p<0.05
are complex mixtures of various substances that are genotoxic and carcinogenic to oral epithelial cells.12 Micronucleus is the result of chromosome changes originating from fragments or whole chromosomes that lag in the anaphase during the break up of chromosomes induced by many genotoxic agents.

Several studies have shown different results regarding the association between cigarette consumption and increasing MN frequencies in buccal mucosa cells.13,14 In this study, the results of the increase of MN frequency was varied between individuals, with an average value of 6.39±3.92 in group A and 6.93±5.82 in group B. The study by Nersesyan showed that smoking induced MN and other chromosomal anomalies in buccal cells. The cytogenetic effects of cigarettes on exfoliated buccal cells are inconsistent, and these findings suggest that this mismatch may partly be due to variations in tar and nicotine levels of cigarettes smoked by participants.

Folic acid is a part of vitamin B which act as an antioxidant. Several studies have shown that folic acid supplementation can reduce the MN frequency in lymphocytes, buccal mucous epithelial cells significantly in high-risk groups (smokers, alcoholics, patients with chronic diseases). Folic acid provided in high dosage in order to protect cells from the occurrence of DNA damage due to the exposure to high oxidants. Supplementation of 2×1,000 mcg and 3×500 mcg of folic acid was provided for 30 days and was shown to decrease the buccal mucosa MN frequency in previous studies.16 Our study used folic acid supplementation once daily of 400 mcg, and 1,000 mcg dose indicates the improvement of MN frequency by 50% between the pre-supplementation and post-supplementation of folic acid. Nevertheless, the MN reduction not synchronized with the average MN frequency of healthy people about 0.5–2.5 MN per 1,000 cells according to the research implemented by Jois et al.17

Several factors that influence the form of anomalies include smoking habits, local and systemic infections, lifestyle, and the other chronic diseases underlying it. In this study, participants had the same risk factors for the emergence of MN based on data obtained through filling out questionnaires in participants at the beginning of the study. Some participants had an adequate to bad oral health index in the two treatment groups. It could be one of the supporting factors for the high frequency of MN in participants. There is a strong relationship between the plaque index and the calculus with an increase in the frequency of micronucleus in the cell oral epithelium. Dental and calculus plaques are places for oral bacteria that produce chronic bacterial infections.12 Chronic infections lead to chronic inflammatory processes that are carcinogenic and are associated with the formation of clastogenic genetic damage in oral epithelial cells.18

**Conclusion**

This study concluded that administration of folic acid at a dose of 400 mcg and 1,000 mcg give similar results to a decrease in the buccal mucosa MN frequency in active smokers.

**Conflict of Interest**

The authors declare no conflict of interests.

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