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**ANALYSIS OF SULFUR VAPOR EXPOSURE TO THE NUMBER OF
MICRONUCLEUS AND ORAL BUCCAL EPITHELIAL MORPHOLOGY**

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ABSTRACT

Background: The sulfur vapor consists of SO₂ and CO₂ which are genotoxins that may cause the damage of DNA to the micronucleus in buccal epithelial cells. Micronucleus is a mass like a nucleus, measuring one-third of the nucleus. DNA damage can also be seen from changes in the morphology of epithelial cells. **Objective:** This study aimed to identify the effect of sulfur vapor exposure on the number of micronucleus and morphology epithelial cells in the oral cavity on the sulfur miner. **Methods:** The method of this study was analytic observational with a cross-sectional approach. The total sample of this study was 24 respondents divided into 2 groups, each group contained 12 respondents. Exfoliated buccal cells were collected by scrapping the buccal mucosa. The specimens stained using Hematoxylin and Eosin. Nucleus and cytoplasmic area were examined using image J 1.40 **Results:** The result showed the average number of buccal mucosa micronucleus on coal miners higher (35,50) than controls (11,58). The result of Independent-measures T-test obtained significant different on the number of micronucleus between sulfur miner and controls ($p=0,000$). The result of Independent-measures T-test on the nuclear area and cytoplasmic area between sulfur miner and controls obtained insignificant different ($p=0,379$ dan $p=0,616$). **Conclusion:** Based on this study can be concluded that sulfur vapor exposure affected on the number of micronucleus on sulfur miners, but did not influence morphology of epithelial cells.

Keywords: Epithel morphology, Micronucleus, Sulfur vapor

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INTRODUCTION

Indonesia is the country with the most volcanoes in the world. One of them is Mount Welirang, located in Pecalukan Village, Prigen District, Pasuruan Regency, East Java Province. Welirang Mountain area is a large sulfur source. This sulfur has been used for years as a livelihood for some residents. Sulfur mine workers have the risk of being exposed to chemical compounds in the sulfur vapor content originating from the mountain crater. These chemical compounds include sulfate gas (S, SO_x (SO₂ and SO₃), and H₂S), fumarole vapor (water vapor (H₂O) and nitrogen gas), carbon dioxide gas (CO₂) and CO, hydrogen chloride, and hydrogen fluoride, which can threaten workers at any time.¹⁻⁵

Sulfur vapor could enter the body through the respiratory tract and oral cavity. The oral cavity is one of the entrances to substances that are harmful to the body and can cause damage to DNA. Sulfur dioxide (SO₂) and carbon dioxide (CO₂) contained in sulfur vapor are genotoxins in the form of free radicals which are included in ROS. ROS can cause damage to DNA. DNA changes due to genotoxic substances can

be seen in buccal mucosa cells in the form of micronucleus.⁶⁻⁹

Micronucleus is a mass located in the cytoplasm and adjacent to the nucleus. Micronucleus structure similar to the nucleus, sized one-third of the nucleus, oval-shaped. Micronucleus is formed due to failure of mitosis, where fragments or whole chromosomes fail to be pulled by spindle threads to opposite poles. DNA damage can be seen from changes in the area of the nucleus and cytoplasm in epithelial cells.^{10,11}

Research on micronucleus and morphological changes in epithelial cells was carried out before, but no studies have examined the sulfur vapor effect to the number of micronucleus and changes in epithelial cell morphology. The purpose of this study was to determine the number of micronucleus and morphology of buccal oral mucosa epithelial cells in sulfur mine workers.^{12,13}

MATERIALS AND METHODS

This type of research was quantitative research. Ethical clearance for the study was obtained from the Ethical Committee Board, Faculty of Dentistry,

Universitas Islam Sultan Agung No. 030/B.1-KEPK/SA-FKG/VIII/2018. The study used an observational analytic method with a cross-sectional approach. Sampling used nonprobability sampling techniques, namely consecutive sampling by selecting according to inclusion and exclusion criteria. The number of the subject was 24 people divided into two groups: sulfur mine workers and residents of Pecalukan Village.

The inclusion criteria in this study were male sex, aged 20-24 years, and work as a sulfur miner at least for 3 years. While the exclusion criteria were subjects who did x-rays for the last 6 months, had systemic diseases, consumed certain drugs, and smoked. The tools and materials needed in this study are a wooden spatula, object-glass, slip glass cover, object glass box, drop pipette, light microscope, informed consent sheet, stationery, fixative solution of methanol-acetic acid (3: 1), distilled water, and hematoxylin-eosin staining.

The research procedure begins with introductions, interviews, and filling out informed consent. Then the subject instructed to rinse the mouth with one 250 cc glass of water to remove debris in the oral cavity. Buccal mucosa scraping was carried out using a wooden spatula. The scrapped epithel staining used hematoxylin-eosin stain.

The stained preparations were observed under a light microscope with a magnification of 400x. Before calculating the micronucleus, epithelial cells must enter the criteria of Tolbert et al. Tolbert et al criteria mentioned micronucleus as which are less than one-third the diameter of the normal nucleus but large enough to be seen both in shape and color, the intensity of the color and texture are similar to the nucleus, texture and focal plane are the same as the nucleus. Micronucleus do not merge with the cell nucleus or overlap with the nucleus. Micronucleus that has been identified according to the Tolbert et al criteria was calculated with the help of a handy counter. The number of micronucleus is calculated per 100 cells. After calculating the micronucleus, nucleus area and cytoplasmic area epithelial cells measured using application Image J 1.40 by using digitalized cursor with an interactive measurement (making an outline at the epithelial membrane and nucleus membrane). The application then will count the area size automatically in square micrometers.

RESULTS

The results of the study found that the average number of buccal mucosa micronucleus in sulfur mine workers was higher than in the control group. The data were normally distributed and homogeneous.

Table 1. Mean of micronucleus number, cytoplasmic area and nuclear area

Group	Mean of micronucleus number	Mean of cytoplasmic area	Mean of nuclear area
Sulfur miner	35,50±13,,3	61.851±15.247	1.140±305
control	11,58±6,87	59.064±11,279	1.024±266

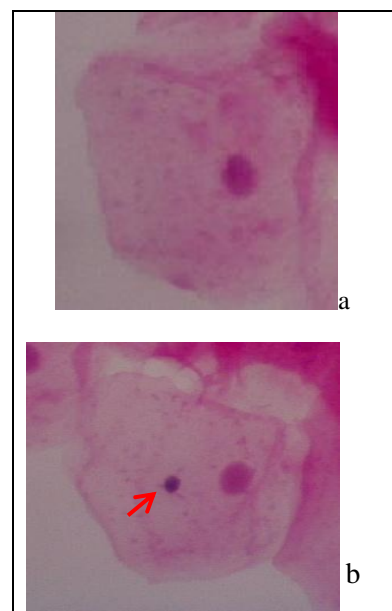


Figure 1. Buccal epithelial cell (magnification 400x): (a). control group, (b) sulfur miner group

The data then analyzed using parametric Independent-measures T-test to determine the difference in the number of micronucleus between groups of sulfur mine workers and control groups. Parametric results of Independent-measures T-test p-value = 0,000 ($p < 0.05$) which means that there was a significant difference between the sulfur mine worker group and the control group.

Table 2. Independent-measures T-test

Group	N	Number of Micronucleus (Sig.)	Nuclear Area (Sig.)	Cytoplasmic Area (Sig.)
Sulfur miner	12	0,000	0,336	0,616
Control	12			

The average nucleus area in the sulfur mine workers group was higher than the control group. The average cytoplasmic area in the sulfur mine workers group was higher than the control group. Data on differences in nucleus area and cytoplasmic area were analyzed using parametric Independent-measures T-

test. The result of Independent-measures T-test on the nuclear area and cytoplasmic area between sulfur miner and controls obtained no significant difference ($p=0,336$ dan $p=0,616$).

DISCUSSION

Belerang mine workers in Mount Welirang have a great potential to be exposed to sulfur vapor because sulfur mine workers do not use personal protective equipment such as masks, gloves and appropriate shoes. It can cause SO_2 and CO_2 in sulfur vapor enter the body through the respiratory and digestive pathways. SO_2 and CO_2 are free reactive radicals. Free radicals derived from oxygen derivatives are called Reactive Oxygen Species (ROS).^{14,15}

This study showed that the number of micronucleus between groups of sulfur mine workers and control groups was significantly different ($p=0,00$). The number of micronuclei is higher at the sulfur miner. It is because SO_2 and CO_2 at the sulfur vapor act as ROS in the body. ROS enters the cell nucleus by damaging the cell membrane. DNA-bound ROS can cause DNA oxidation and nucleotide base modification, causing gene imbalance and making chromosomes unstable. One of the instabilities on the chromosome can cause an acentric chromosome.^{11,15,16} Micronuclei (Mni) appear in the cell cytoplasm and originate from acentric chromosome fragments (particularly from chromosomes 1, 9 or 16), acentric chromatid fragments or whole chromosomes (generally sex chromosomes, especially the X chromosome) and fail to be included in the daughter nuclei at the completion of telophase during mitosis. At that time, the remaining chromosomes will be formed like the nucleus but with smaller size. This nuclei is called Micronuclei.¹⁷

Observations suggest that oncogenes harboring in MN contribute to the invasive characteristics of tumor cells.¹⁸ ROS display on DNA can lead to premalignant and malignancy. However, there has been no research on the average indicator of the number of micronucleus in a cell development to the premalignant lesions and malignant lesions. Khanna et al said that the average number of micronucleus in the premalignant and malignant lesions was higher than the control group.^{19,20}

It has been postulated that exfoliated buccal mucosa cells have a high predictive value for the early detection of carcinogenesis since the majority of human tumours are of epithelial origin. Several confounding factors (lifestyle habits (smoking, alcohol, etc.), age, and exposure to mutagen agents such as sulfur vapor) may modulate MN formation and frequency.¹⁷ This study used buccal mucosa epithelial cells of the oral cavity to examine micronucleus. The use of oral buccal epithelial cells is a non-invasive method. The micronucleus can be seen in primary lymphocyte cells, liver, vagina, and buccal mucosa epithelial cells of the oral cavity.²¹

Exfoliative cytopathology is a reliable way of taking epithelial cells on the mucosal surface of the oral cavity. Exfoliative cytopathology aims to detect and diagnose benign or malignant lesions. According to the American Journal of Dental Association (ADA) exfoliative cytopathology is an examination performed by a dentist if there are suspected oral lesions. Exfoliative oral cytology can early detect 60% of oral cavity cancers and 80% of deaths can be prevented.^{19,22-24} Changes in the morphology of epithelial cells in the form of changes in the size and shape of the nucleus of the cytoplasm can be used as an indicator of the presence of dysplastic or neoplastic lesions.²⁰

According to Singh et al. 2014, cell changes were characterized by morphological changes such as hyperchromatic, nucleus enlargement and increased mitosis. Increasing the size of the nucleus and decreasing the size of the cytoplasm are a characteristic of malignancy. In this situation, the ratio of the nucleus and cytoplasm can reach 1: 1, whereas in the normal state of the nucleus and cytoplasm it has a comparison of 1: 4 or 1: 6.^{21,25}

Based on data analysis, the differences in the cytoplasmic area were not significant between the sulfur mine workers group and the control group ($p = 0.379$). The area of the nucleus between the sulfur mine workers group and the control group also showed a nonsignificant difference ($p = 0.616$). This study is in line with Nivia et al's study, namely differences in nucleus area and cytoplasmic area seen only in premalignant and malignant lesions. Epithelial cell undergoing malignant transformation typically shows a reduction in cytoplasmic and nucleus area.¹³ It can be assumed that sulfur in welirang was at low doses and doesn't have a direct effect on the formation of malignancy or dysplastic lesion.²⁶ It is concluded that Sulfur vapor exposure affected on the number of micronucleus on sulfur miners, but did not influence morphology of epithelial cells.

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