

## DIRECT MN TEST ON PERIPHERAL BLOOD TO DETECT CHROMOSOMAL BREAKAGE: APPLICATION IN SMOKERS

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### Abstrak

Tujuannya adalah untuk meneliti kerusakan kromosom pada sel mononuklir darah perokok. Dengan tujuan tersebut telah dilakukan skrining mikronukleus pada sampel darah-tepi perokok. Sampel yang berasal dari perokok yang sakit tidak diikuti sertakan. Dari tiap sampel diperiksa 500 lekosit mononuklir yang mengembang dengan menggunakan mikroskop cahaya, dengan pembesaran 400x. Dibuat tabulasi frekuensi distribusi subjek dengan mikronukleus 0, 1, 2, 3, 4, dan 5, menurut umur dan keadaan subyek. Dari 102 sampel, 5 tidak diikutsertakan, dan hanya 97 yang dianalisis. Terdapat peningkatan mikronukleus berturut-turut pada 12,8% perokok normal yang bertempat tinggal di daerah tak terpolusi, 12,9% perokok dengan hipertensi yang bertempat tinggal di daerah tak terpolusi, 33,3% perokok normal yang bertempat tinggal di daerah terpolusi, dan 25% perokok dengan hipertensi yang bertempat tinggal di daerah terpolusi. Dengan demikian, terdapat kecenderungan peningkatan mikronukleus pada perokok dalam usia produktif, penderita hipertensi dan orang yang bertempat tinggal di daerah terpolusi.

### Abstract

The purpose was to assess chromosomal damage in blood mononuclear cells of smokers. Smoker's peripheral blood samples were screened for micronuclei. Samples from smokers who had an illness were excluded. From each sample, 500 swelled mononuclear leucocytes were screened using a light microscope, with 400x magnification. Frequency distribution of subjects having 0, 1, 2, 3, 4, and 5 micronuclei (MN) according to age and condition were tabulated. From the 102 samples, 5 were excluded, and only 97 were analyzed. There was an increase in MN count in 12.8%, 12.9%, 33.3%, and 25% of normal smokers living in unpolluted area, hypertensive smokers living in unpolluted area, normal smokers living in polluted area, and hypertensive smokers living in polluted area, respectively. Therefore, there was a tendency of increasing MN count in smokers in the productive age group, hypertensive people, and people living in polluted area.

*Keywords: Micronucleus, mononuclear, leucocytes, hypotonic, clastogen*

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### Introduction

Smoking was associated with increased frequency of chromosomal damage that had been detected in exfoliated cells in sputum<sup>1</sup>, and uterine cervix<sup>2</sup>. Detection of chromosomal damage in blood can be done by chromosomal analysis, sister chromatid exchange analysis, or a simpler method, i.e. micronucleus (MN) tests on binucleated lymphocytes<sup>3</sup>.

However, micronucleus test on binucleated lymphocytes needs lymphocyte culture and cytochalasin B, that need high cost and at least three days for culture<sup>3</sup>. Recently, we have developed a very simple and cost effective method to detect chromosomal damage in blood mononuclear cells by screening those cells for micronucleus (MN)<sup>4</sup>.

It is not known, whether smoking also causes chromosomal damage in blood mononuclear cells. Therefore, in this study, we used the direct micronucleus test on blood mononuclear cells to assess chromosomal damage in blood mononuclear cells of smokers.

### Methods

#### *Samples and data*

A total of 102 blood samples were collected from smoking blood donors who came to the Indonesian Red Cross, Central Jakarta. The samples were collected from 16th March through 3rd April 1998. From each donor, 3 ml of blood were collected from the blood sac tubing. Data from blood donors were collected from donor's

records. The data collected were sex, age, illness, blood pressure, and address. Data of polluted areas in Jakarta were received from the urban recitation and environment office, Jakarta<sup>5</sup>. Samples from donors who suffered from illnesses (allergy, asthma, diabetes, etc.) were excluded.

#### Procedure

Blood were placed in heparinized vacutainer tubes, and the erythrocytes were allowed to be gravity separated from the plasma. Soon after the plasma was separated, it was mixed with aqua bidestilata (plasma: aqua= 2:1, 3:1, and 4:1), and each diluted plasma was quickly smeared on-to a glass slide, and fixed briefly by modified Carnoy solution (methanol: glacial acetic acid = 9:1). The slides were stained using Giemsa solution (pH 6.5) for 5 minutes, air dried, and mounted in Canada balsam<sup>4</sup>. From each sample, three slides were made.

#### Analysis

The slides were examined under a light microscope, using a 400x magnification. A number of 500 swelled mononuclear cells were screened, and the number of cells containing a micronucleus was counted. For each subject, the number of micronuclei/500 cells was noted. Subjects with micronucleus count > 1/500 mononuclear cells were regarded as having an increase in micronucleus count<sup>6</sup>. The data of micronucleus count/500 cells were classified according to donor's condition (hypertensive, and living in a polluted area), and age, and tabulated. Micronucleus count was analyzed according to donor's condition and age, whether there are differences between age groups and between several conditions, respectively. The differences were analyzed using Kruskal-Wallis test using a computer software SPSS 10.0.1 for Windows 98/2000/NT<sup>7</sup>.

## Results and Discussion

From the 102 subjects, 5 were excluded, and only 97 subjects were analyzed. From the 97 subjects, 96 were males, and only 1 was a female. The frequency distribution of normal smokers living in unpolluted area who had 0, 1, 2, 3, 4 and 5 micronuclei (from the 500 cells screened) according to age is shown in table 1.

**Table1. Frequency distribution of normal smokers living in unpolluted area who had 1, 2, 3, 4, and 5 micronuclei according to age**

Age	Frequency distribution of subjects with MN count of						Total
	5*	4*	3*	2*	1	0	
<24	-	-	-	-	-	2	2
25-34	-	-	1	2	3	14	20
35-44	1	-	-	1	3	6	11
45-54	-	-	-	-	-	5	5
>55	-	-	-	-	-	1	1
Total	1	-	1	3	6	28	39

\*increased micronucleus count

Although statistical analysis showed that there was no significant difference in MN count between age groups, our result showed that in productive age groups (between 25-44 years), there was an increase in MN count in 2 subjects. This fact might be due to the longer exposure compared to younger age groups. In the older age groups, there was no increase in micronucleus count. We supposed that this fact was due to older people's habit i.e. giving more attention to their health, and therefore reduce smoking. However, we could not prove our assumption, since in this study we were not allowed either to interview the donors or to let the donors fill in a questionnaire, in order not to disturb the donors. Therefore, the data were taken from the donor's record, and we could not obtain any data concerning the number of cigarettes consumed/day, or the duration of smoking. Furthermore, in older age groups, our results was in line with a study that showed a lower response to mutagenic substance in older mice compared to young mice<sup>8</sup>.

Many substances have been classified as clastogen (an agent that causes chromosomal breakage), and many others are not known yet, but still have the potential to be clastogen. Therefore, the conditions causing ingestion/absorption of substances, not proven as nonclastogens were categorized as groups which should be excluded. As examples for nonclastogens are probably certain components of drugs, consumed by subjects with a disease. Furthermore, to minimize the effect of possible intake of antihypertensive drugs in hypertensive subjects and inhalation of pollutants in subjects living in a polluted area, subjects were classified according to their blood pressure (hypertensive and normal) and their address (living in a polluted or unpolluted area). The frequency distribution of subjects having 1, 2, 3, 4, and 5 micronuclei according to their condition is shown in Table 2.

**Table 2. The frequency distribution of subjects having 1, 2, 3, 4, and 5 micronuclei according to their condition**

MN count /500 mono-nuclear cells	Frequency distribution of subjects			
	Living in unpolluted area		Living in polluted area	
	Normal	Hypertensive	Normal	Hypertensive
5*	1	-	-	1
4*	-	1	1	1
3*	1	1	-	1
2*	3	2	4	0
1	6	12	3	4
0	28	15	7	5
Total	39	31	15	12

\*increased micronucleus count

Our results showed that in smokers there was an increase in MN count in 5 of the 39 (12.8%), 4 of the 31 (12.9%), 5 of the 15 (33.3%), and 3 of the 12 (25%) of normal smokers living in unpolluted area, hypertensive smokers living in unpolluted area, normal smokers living in polluted area, and hypertensive smokers living in polluted area, respectively. It seemed that there was a tendency of increasing MN count in smokers, hypertensive people, and people living in polluted area. However, statistical analysis showed that there was no significant difference in MN count between those various conditions.

Micronucleus can only be seen after a cell proliferates<sup>9</sup>. Cells that exfoliate are always proliferating, and screening micronuclei in those cells is a tool to assess chromosomal breakage<sup>1,2,10</sup>. However, mononuclear leucocytes are only proliferating when they have encountered an antigen. Therefore, screening of micronuclei directly in mononuclear leucocytes is supposed to be valuable only in detecting clastogen exposure, after a certain time following the exposure, or in chronic exposure, e.g. in smokers, people taking long term medication, or living in a polluted area.

### Conclusion

There is a tendency of increasing MN count in smokers in the productive age group, hypertensive people and people living in polluted area.

### Acknowledgements

The authors would like to thank the Chief and paramedics of the Indonesian Red Cross, Central Jakarta, and all the donors who have participated in this study. This study was funded by the Risbin ptekdok II research grant from the Indonesian Institute of Sciences (LIPI).

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