

Original Articles

The Effect of Perchloroethylene (PCE) Exposure Toward Frequency of Micronucleus Formation in Buccal Mucosal Epithelia of Dry Cleaning Employees

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Abstract

Background: Perchloroethylene is one of the main ingredients used in the dry cleaning industry. Continuous inhalation of Perchloroethylene particles can cause genetic damage that can be marked by the appearance of micronucleus. Micronucleus formation of excessive buccal mucosal is one of the early signs of malignancy in the oral cavity.

Objective: To determine the effect of Perchloroethylene exposure toward frequency of micronucleus formation in buccal mucosal epithelial of dry cleaning employees in Banyumas Regency.

Materials & methods: This study uses cross-sectional design with primary data that is the preparation of buccal mucosal smear from 22 dry cleaning employees as exposed group and 22 subjects are not exposed to Perchloroethylene as control group. The buccal epithelial cells are collected using a spatula and then smeared on an object glass and colored using Giemsa. Micronuclear frequencies are calculated per 1000 cells using a light microscope.

Results: The difference of micronucleus frequency between two groups ($p = 0.000$) is obtained. The duration of work has correlation with medium correlation strength to the forming frequency of micronucleus formation, whereas age has strong correlation to the forming frequency of micronucleus formation. The correlation between age and work duration with the forming frequency of micronucleus formation is significant ($p = 0.000$ and $p = 0.07$)

Conclusions: There is influence of Perchloroethylene exposure toward frequency of micronucleus formation. The duration of work and age are significantly correlated with the forming frequency of micronucleus formation.

Keywords: Perchloroethylene, micronucleus, dry cleaning.



DOI: <https://doi.org/10.3329/jom.v20i2.42003>

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Received: 17 January, 2018;

Accepted: 09 June, 2018

Introduction:

Perchloroethylene (PCE) or tetrachloroethylene, is a chlorocarbon group with chemical formula C_2Cl_4 that is colorless, non-flammable and unstable.¹ Its use has been known since 1930 as the main ingredient of cleaning clothes in the dry cleaning industry.² Due to its very unstable nature,

PCE easily escapes into the environment and gets carried into the airway through the inhalation process.³

Acute PCE exposure can cause conditions such as upper airway and eye irritation, renal dysfunction, and neurological effects such as changes in posture, dizziness, headache, insomnia and fainting. While the major effects due to chronic PCE exposure in humans include liver damage, kidney, immune and hematologic and reproductive system.¹

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United States Environmental Protection Agency (US.EPA) determines the maximum concentration and dose for PCE exposure in one day is 0.04 mg/m^3 and 0.006 mg/kg/day .⁴

Some research has been done on PCE mentioned that repeated exposure over 70 mg/m³ (4 10 p.pm) in a day can increase the risk factors of genetic defects that trigger cancer.^{5,6}

Genetic damage caused by PCE can be detected through nucleus anomaly tests. The nucleus anomaly test is mostly used by researchers because it is easy to do and the cost is cheaper than other tests. Parameters that are easily seen if there is genetic damage due to PCE exposure are micronucleus.

Micronucleus is an abnormal cell nucleus with a diameter of 1/3-1/6 of the normal nucleus diameter and is located next to the normal nucleus. Micronucleus is formed by chromosomal damage during the mitosis process. Micronucleus is a biomarker that can be detected due to exposure to genotoxic material as well as a sign of the initial mechanism of carcinogenesis.⁷ Everatt *et al* mentioned that the micronucleus frequency is increasing in the PCE exposed group compared with the non-group.⁵

Micronucleus evaluation can be performed in erythrocyte, lymphocytes and buccal mucosal epithelium. Bucal cells are the first barrier for the inhalation or ingestion route and are capable of metabolizing proximate carcinogens to reactive products.¹⁴ Evaluation of buccal mucosal epithelial smears is more widely used today because of it is an easier, quicker and cheaper procedure.⁸

Materials and methods:

Study population

This research uses cross-sectional design with primary data that is buccal mucosal smear form 44 subjects. The study protocol was reviewed and approved by the Ethical Committee of University of Muhammadiyah Purwokerto in Indonesia. The subjects are divided into two groups, 22 dry cleaning employees as exposed groups and 22 subjects were not exposed to PCE as control group. Subjects with smoking habits, drinking alcohol, history of genetic diseases, Alzheimer’s, neoplasia and metabolic syndrome like diabetes mellitus and hypertention and subjects who are undergoing treatment with radiation were excluded from the study.

Sample collection

Subjects were asked to rinse the mouth with water and then the buccal epithelial cells are collected using a spatula and then smeared on an object glass and fixed in methanol for 15 minutes. The fixed slides were allowed tro dry, and then colored using Giemsa stain, diluted with phosphate bufer in the ratio 1:6 for 10 minutes. Micronucleus frequencies are calculated per 1000 buccal cell using a light microscope with a 400x zoom with reference to the micronucleus criteria belonging to Bolognesi *et al*.⁷

Statistical analysis

The MN forming frequency data in the dry cleaning employees group and control group were analyzed using unpaired t-test, whereas the relationship between age and duration of work or duration of PCE exposure with micronucleus frequency will be analyzed using Pearson correlation test. A value of p <0.05 is used to determine the level of significance.

Results:

The result of data analyze showed that there was significantly difference between exposed group and control group. Control group showed lower mean micronucleus frequencies compared to exposed group.

The average and standard deviations of micronuclear frequencies of the two groups’ are shown in the Table I and figure 1. Standard deviation is the average of each data difference against the overall average. The average frequency of micronucleus in dry cleaning employees is higher than control group. The mean number of micronucleus formation was 14.5±6.25 in the dry cleaning employee group and 4.5±1.26 in control group.

Table I. The unpaired t-value results against the dry cleaning and control groups

	N	Average ± SD	P
Dry cleaning employees	22	14.5 ± 6.25	0.000
Control	22	4.5 ± 1.26	

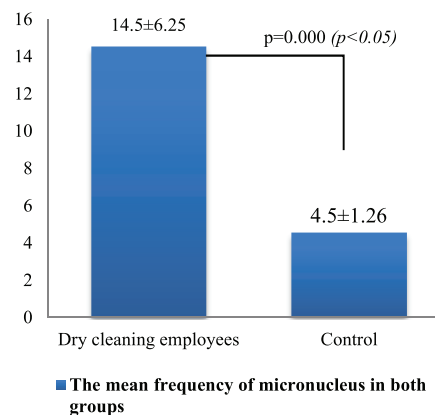


Figure 1. The mean frequency of micronucleus in both groups

The difference between the two groups is significant due to the significance of p = 0.000 (p <0.05), it is indicating that *Perchloroethylene* exposure on dry cleaning employees may increase the frequency of buccal mucosal formation.

Photomicrographs of micronucleus are shown in figure 2 and 3. The analysis is followed by Pearson correlation test to determine the correlation between age and duration of work (exposure duration) on forming frequency of micronucleus from dry cleaning employees.

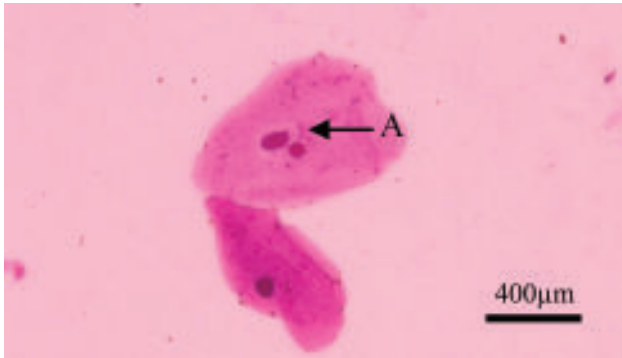


Figure 2. Photomicrographs of buccal epithelial cell with micronucleus (Giemsa stain, 400x) (A).

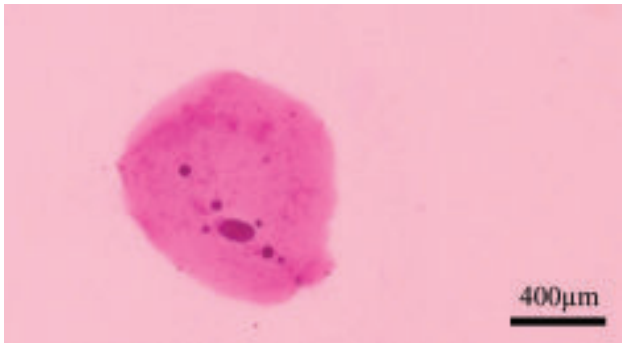


Figure 3. Photomicrographs of buccal epithelial cell with multiple micronucleus (Giemsa stain, 400x) (B).

From the Pearson correlation test results obtained p value = 0.07 ($p < 0.05$), this indicates that the correlation between the duration of exposure (duration of work) with the forming frequency of micronucleus in dry cleaning employees is significant. Pearson correlation value is 0.557, which shows a positive correlation with moderate strength.

The correlation between age and forming frequency of micronucleus is also significant because $p = 0.000$ ($p < 0.05$), with Pearson correlation value is 0.703, which means there is a strong positive correlation that can be seen in tables II and III.

Table II. Pearson correlation test results of duration of work on the forming frequency of micronucleus

		Duration of work	Micronucleus Frequency
Pearson	Duration of work	1	0.557
	Micronucleus Frequency	0.557	1
P			0.007

Table III. Pearson correlation test results of age on forming frequency of micronucleus

		Age	Micronucleus Frequency
Pearson	Age	1	0.703
	Micronucleus Frequency	0.703	1
P			0.000

Discussion:

The results show that micronucleus is not only found in the dry cleaning employee, but also in the control group. The average micronucleus frequency measurement results lower in the control group (4.5 ± 1.26) compared with the dry cleaning group (14.5 ± 6.25). This is reinforced by unpaired t-test results indicating a significant difference between the average forming frequencies of micronucleus from the two groups of ($p < 0.05$). Thus the results of this study are in accordance with the hypothesis that *Perchloroethylene* exposure can increase the forming frequency of buccal mucosal in micronucleus from dry cleaning employees.

The results of this study indicate that *Perchloroethylene* exposure affects the increased forming frequency of micronucleus in the buccal mucosal epithelial from dry cleaning employees. This is consistent with the study of Everatt *et al.*⁵ which states that *Perchloroethylene* exposure to dry cleaning employees with smoking habits has higher forming frequency of micronucleus (12.61 ± 0.39) than the control group with smoking habit (2.79 ± 0.16). The difference between the two groups is significant because it has a significance value of $p < 0.001$.

Perchloroethylene exposure to dry cleaning employees occurs through inhalation process. *Perchloroethylene* particles are released into the environment from the washing process will enter through the inhalation route and then attached to the buccal mucosal as the first barrier to exposure to genotoxic substances.⁹

Perchloroethylene (PCE) contains several substances that are genotoxic. In the body, the substance will be described by cytochrome p-450 enzyme through oxidation reaction to produce reactive metabolite. The metabolite will then enter the bloodstream and trigger a chromosome chain breakdown.¹⁰ The fragments will then be left in one of the equatorial fields during the phase of anaphase division of mitosis core membrane will then enclose the chromosomal fracture in the phase of the telophase, so that a new nucleus is formed which is smaller and separate from the main core. This small nucleus is called micronucleus.^{7,8,11,13}

One of the manifestations from genotoxic effects occurring on the buccal mucosal is the formation of micronucleus.^{9,11}

The dry cleaning officer who participated in the study had an average working life of 4-5 years. The duration of *Perchloroethylene* exposure to dry cleaning employees associated with duration of employment is also associated with the emergence of micronucleus, this can be evidenced from the results of correlation test between the duration of work with the frequency of micronucleus formation which has a correlation value of 0.557 with significance $p = 0.007$ ($p < 0.05$). This is consistent with the research from Calvert *et al.* (2011) that states *Perchloroethylene* exposure in dry cleaning employees who work ≥ 5 years has high micronucleus frequency.

In this study, age is also positively correlated with frequency of micronucleus formation with correlation value of 0.703 and significance $p = 0.000$ ($p < 0.05$). The result is consistent to a study by Thomas *et al.*¹² on micronucleus comparisons between young adults, Down syndrome patients and the elderly. The results show a significant increase in micronucleus to the elderly compared with the young adult group ($p < 0.05$)¹². This happens because in old age there is a change of genotype of the cell structure and nucleus. This change will lead to an increase in the incidence of genetic instability that is often associated with reduced body capacity to repair cellular and DNA damage.¹²

This study shows that acute exposure to *Perchloroethylene*, which is one of the genotoxic materials, can induce the formation of buccal mucosal epithelial micronucleus, so that the main ingredient in this dry cleaning industry can be categorized as one of the carcinogenesis materials that can potentially cause cancer in humans, although the process is still unknown clearly.

Conclusions:

There is an influence of *Perchloroethylene* exposure toward micronucleus formation on buccal mucosal epithelial of dry cleaning employees in Banyumas Regency. The longer the duration of exposure or working time, the higher the frequency of micronucleus formation.

Acknowledgement:

To Faculty of Medicine University of Muhammadiyah Purwokerto, Indonesia who had to support the making of this article and allowed the utilization of its Pathology Clinic Laboratory for this study until the finish.

Conflict of interest: None.

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