

Evaluation of Micronuclei in Occupational Hazard Cases using Acridine Orange Stain

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ABSTRACT

Aim: The aim of this prospective study was to evaluate micronuclei in automobile painters and welders with and without the effect of habits such as smoking, tobacco chewing and alcohol in these individuals using Acridine Orange stain.

Materials and Methods: The present study was carried out in 40 males in the age group of 18-45 years exposed to occupationally hazardous chemicals in automobile painters and welders with and without any history of smoking, tobacco chewing and alcohol. Slide for each participant was clearly labeled to avoid losing or mixing up information. Before collection of sample patients were asked to rinse their mouth thoroughly with water to remove any unwanted food debris. Exfoliated buccal cells were obtained by rolling the cytobrush against the buccal mucosa in a gentle motion. The cells were smeared over a pre cleaned coded microscopic slide. Smears were then fixed with 95% ethanol for 30 min. before they can be stained. Fixed smears were stained with Acridine orange. Micronuclei cells were counted in 1000 intact epithelial cells and were scored at 100x magnification using oil immersion with a fluorescent microscope.

Results: In our study we included 40 individuals divided into 4 groups. The micronuclei were counted in each slide and difference between highest mean (12.05) and lowest mean for control group (2.88) and Standard deviation (1.85) was used to calculate using the ratio of difference between 2 means (worst and best) to the S.D.

The mean of micronuclei was higher in individuals involved in occupations like welding, automobile painting and in the individuals without occupations but having habit of smoking, tobacco chewing and alcohol consumption as compared to the control group.

Conclusion: In our study we concluded that the micronucleus assay provides an efficient method to detect and subsequently score micronuclei present in buccal mucosal cells, which indicates an early evidence of genotoxic damage.

A significant correlation was established between occupational hazards and micronuclei frequencies. Workers in many occupational settings are exposed to certain genotoxic agents. These workers may not be aware that they have been exposed to genotoxic agents.

Therefore, there is a need to educate those who work with such occupational settings about the potential hazard of occupational exposure and the importance of using proper protective measures.

Keywords: Micronuclei, occupational hazards, acridine orange

INTRODUCTION

Occupational health deals with all aspects of health and safety in the workplace and has a strong focus on primary prevention of hazards. The health of the workers has several determinants, including risk factors at the workplace leading to cancers. Millions of workers in a variety of occupational settings have the risk to be exposed to hazardous substances. They can be present in the occupational environment in the form of gases, vapors, fumes and particles.¹

Health risk associated with different labors is related to contact with corrosive, infectious, carcinogen, cytotoxic, mutagenic, or genotoxic agents. Research around genetic toxicology and risk assessment or workplace exposure is important since exposure to several hazardous agents is common and can aid in health issues.²

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Hazardous chemicals can enter the body by three routes: oral and respiratory routes and contact with skin and mucosal tissue. Adverse effects of these chemicals depend on the

dose, route, and type. Buccal cells form the first barrier for the inhalation or ingestion route and are capable of metabolizing proximate carcinogens to reactive products. About 92% of human cancers are derived from the external and internal epithelium, that is, the skin, the bronchial epithelium, and the epithelia lining the alimentary canal. Therefore, it could be argued that oral epithelial cells represent a preferred target site for early genotoxic events induced by carcinogenic agents entering the body via inhalation and ingestion.³

To evaluate genotoxic damage, the methodology frequently used is expensive and complicated and usually involves invasion; opposite to this, the micronuclei test (MN test) performed in buccal mucosa cells is a precise, inexpensive, noninvasive, and easy method for measuring DNA damage and cell death in the oral epithelium not requiring cell cultures.⁴

The presence of increased frequency of micronuclei within the cytoplasm of cells is indicative of chromosome loss or fragmentation, which is a sequelae of genotoxic damage. The buccal cell micronucleus (Q2) is defined as the microscopically visible, round or oval cytoplasmic chromatin mass next to nucleus. Micronuclei originate from aberrant mitosis and consist of acentric chromosomes, chromatid fragments or whole chromosomes that have failed to be incorporated in the daughter nuclei during mitosis.⁵

The inhalation of toxic fumes from occupations such as welding has been reported to induce genotoxicity in the individuals involved in these occupations.⁶

Along with this carcinogenic products of smoking and tobacco chewing are believed to be responsible for the induction of micronuclei.⁷

The present study was aimed to evaluate the number of micronuclei in occupations like welders and automobile painters using Acridine Orange stain and to evaluate the cumulative effect of habits like smoking, tobacco chewing and alcohol in these individuals.

MATERIAL AND METHODS

The study had been approved by the Ethical Committee (PUIEC201202-III-008) of the dental institute. Ethical clearance from PUIEC was sought and there were no ethical issues in the study. As per International standard and University standard, patient’s written consent has been collected and preserved by the author(s).

Subjects and Sample Collection:

Group 1A- comprises of welders with history of habit like smoking, tobacco chewing, alcohol. Group 1B- welders without any history of habit like smoking, tobacco chewing, alcohol. Group 2A- automobile painters with history of habit like smoking, tobacco chewing, alcohol. Group 2B- automobile painters without any history of habit like smoking, tobacco chewing, alcohol. Group 3 includes persons with habit of smoking, tobacco chewing, alcohol without involvement in any above mentioned occupation. Group 4 includes control group i.e. person without any history of habit and above mentioned occupation.

Table 1: Intragroup comparison of micronuclei in welders with and without habit

	Mean	Std. Deviation	Std. Error	Significance
With Habit (Ia)	3.40	2.073	0.927	0.001 (significant)
Without Habit (Ib)	1.40	0.504	0.509	

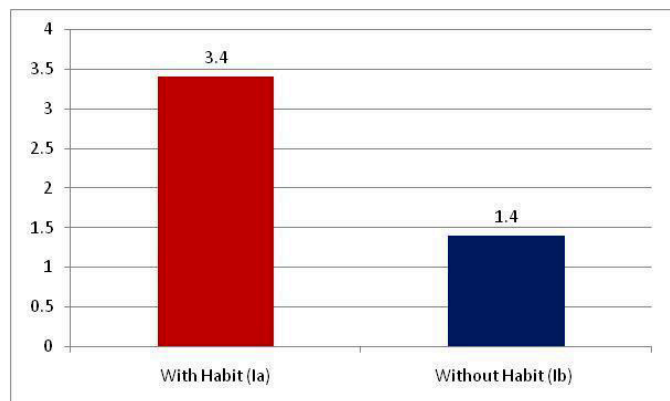


Fig. 1: Intragroup comparison of micronuclei in welders with and without habit

Table 2: Intragroup comparison of micronuclei in automobile painters with and without habit

	Mean	Std. Deviation	Std. Error	Significance
With Habit (IIa)	3.00	1.581	0.707	0.021 (Significant)
Without Habit (IIb)	1.60	0.546	0.374	

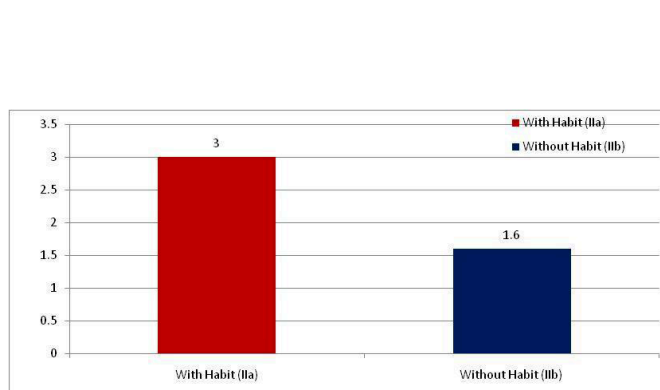


Fig. 2: Intragroup comparison of micronuclei in automobile painters with and without habit



Slides for each participant will be clearly labeled to avoid losing or mixing up information. Before collection of sample patients will be asked to rinse their mouth thoroughly with water to remove any unwanted food debris. Exfoliated buccal cells will be obtained by rolling the cytobrush against the buccal mucosa in a gentle motion. The cells will be smeared over a pre cleaned coded microscopic slide. Two smears will be made from each subject.

Fixation of the smear: Smears will be then fixed with 95% ethanol for 30 min. before they can be stained.

Staining of the smear: Fixed smears will be stained with Acridine Orange.

Observation under the Microscope: Micronuclei cells will be counted in 1000 intact epithelial cells. Micronuclei will be scored at 100x magnification using oil immersion with a fluorescent microscope.

RESULTS

In our study we included 40 individuals divided into 4 groups. The micronuclei were counted in each slide and difference between highest mean (12.05) and lowest mean for control group (2.88) and Standard deviation (1.85) was used to calculated and (Q 3) d is ratio of .;difference between 2 means (worst and best) to the S.D.

Intragroup comparison between welders with and without habit was made and the mean number of micronuclei were significantly higher in welders with habits as compared to subjects without habit when analyzed using the independent t test as shown in table 1 and figure 1.

Intragroup comparison between automobile painters

Table 3: Intergroup comparison of micronuclei in subjects with habit of smoking, tobacco chewing and alcohol consumption (group III) with the control group (group IV)

	Mean	Std. Deviation	Std. Error	Significance
With Habit (III)	2.70	1.159	0.366	0.001 (Significant)
Without Habit (IV)	0.00	0.00	0.00	

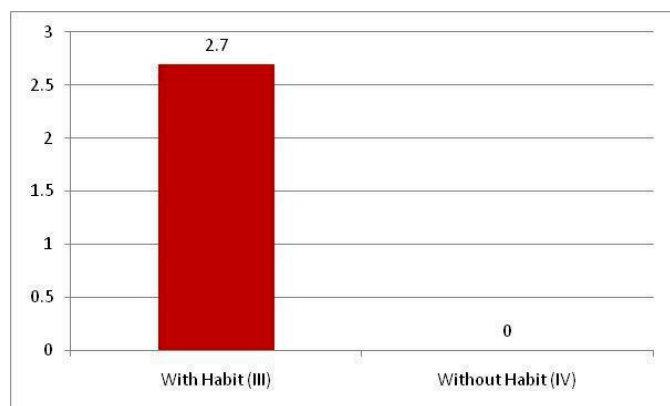


Fig. 3: Intergroup comparison of micronuclei in subjects with habit of smoking, tobacco chewing and alcohol consumption (group III) with the control group (group IV)

with and without habit was made and the mean number of micronuclei were significantly (Q4) higher in automobile painters with habits as compared to subjects without habit when analyzed using the independent t test as shown in table 2 and figure 2.

Significant results were also shown when comparison made between individuals with history of habit of smoking, tobacco chewing and alcohol consumption with the control group shown in table 3 and figure 3.

DISCUSSION

Micronucleus is defined as the microscopically visible, round or oval cytoplasmic chromatin mass next to nucleus. Micronucleus originate (Q5) from aberrant mitosis and consist of acentric chromosomes, chromatid fragments or whole chromosomes that have failed to be incorporated in the daughter nuclei during mitosis.

The term micronucleus was first proposed in early 1970's by Schimdt, Boller and Heddle who demonstrated it as a reliable indicator of genotoxic potential. To evaluate genotoxic damage, the methodology frequently used is expensive and complicated and usually involves invasion; opposite to this, the micronuclei test (MN test) performed in buccal mucosa cells is a precise, inexpensive, noninvasive, and easy method for measuring DNA damage and cell death in the oral epithelium not requiring cell cultures. The presence of increased frequency of micronuclei within the cytoplasm of cells is indicative of chromosome loss or fragmentation, which is a sequelae of genotoxic damage.

In a study conducted by Bhat S et al (2015) included 20 patients with history of smoking cigarettes for five years or more and 20 healthy individuals with no history of habits were selected as the control group. A significantly higher number of micronuclei were seen for smokers in comparison to control group.

In another study conducted by Farha A. Ali Shafiet al (2017) included 24 male participants who were residing in Kufa near cement Factory. A significantly higher number of micronuclei were seen for smokers in comparison to non-smokers.

In our study we included 40 male participants involved in various occupations like welding, automobile painting, individuals having habit of smoking, tobacco chewing and alcohol consumption. A significantly higher number of micronuclei were seen in persons involved in the mentioned occupation and the individuals having habit of smoking, tobacco chewing and alcohol consumption as compared to control group.

Therefore our results demonstrated that Micronuclei assay performed in exfoliated buccal mucosa cells can be a methodology to measure the potential risk in the individuals exposed to various carcinogenic agents.

CONCLUSION

In our study we concluded that the micronucleus assay provides an efficient method to detect and subsequently score micronuclei present in buccal mucosal cells, which indicates an early evidence of genotoxic damage.

A significant correlation was established between occupational hazards and micronuclei frequencies. Workers

in many occupational settings are exposed to certain genotoxic agents. These workers may not be aware that they have been exposed to genotoxic agents.

Therefore, there is a need to educate those who work with such occupational settings about the potential hazard of occupational exposure and the importance of using proper protective measures.

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