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Research Article

Micronucleus assay on buccal cells: an indicator of DNA damage due to formaldehyde exposure in anatomy dissection labs

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ABSTRACT

Background: The occupational exposure to formaldehyde (FA) can lead to various hazards ranging from allergic reactions to genetic damage. Workers of Anatomy lab are at a higher risk of having the hazardous effects of FA. Micronuclei (MN) appear in the cells due to chromosome breakage and dysfunction of the mitotic apparatus which are the indicators for the DNA damage. The present study was carried out to detect the DNA damage in people exposed to FA using buccal cell MN Assay by measuring the MN frequency in buccal cells with respect to the duration of exposure.
Methods: Thirty male workers of Anatomy labs of different medial colleges in Bangalore were included in the study**.** Thirty people with no FA exposure were considered as comparison group. Buccal cells were scraped from the cheek and slides were prepared. A total of 1000 cells were counted for the presence of MN after staining with Geimsa solution.
Results: There was a significant increase in the frequency of MN in both buccal cells ($p<0.001$). A positive correlation was found between the years of exposure and frequency of MN in buccal cells ($r=0.5$, $p=0.03$).
Conclusion: This study highlights that there is a significant DNA damage in people exposed to formaldehyde which is proportional to the duration of exposure.

Keywords: Formaldehyde, Buccal cells, Micronucleus, Anatomy lab

INTRODUCTION

Formaldehyde (FA) is most simple and reactive of all aldehydes, is colourless and readily polymerizing gas at room temperature.[1] It is commonly used preservative in medical laboratories and mortuaries. The occupational exposure to FA can lead to various hazards ranging from allergic reactions to genetic damage.

Inhalation is one of the common routes of exposure especially due to occupations involving FA vapour especially in Anatomy and Pathology laboratories. FA is an irritant and allergen causing occupational asthma, occupational dermatitis, conjunctival and mucosal irritation especially in the respiratory tract.[2]

The International Agency for Research on Cancer (IARC) has classified FA as a human carcinogen that can cause nasopharyngeal carcinoma and also found a strong evidence for causal association between leukemia and occupational exposure to FA.[1] Studies also have shown that long term exposure can lead to the development of pancreatic cancer. Exposure to FA is significantly associated with delayed conception. Associations

between exposure to FA or to organic solvents and endometriosis, spontaneous abortions and salpingo-oophoritis are also reported.[3]

FA induces Deoxyribo Nucleic Acid (DNA) protein cross-links in mammalian cells in vitro and in vivo. In addition to that, it induces DNA single-strand breaks, chromosomal aberrations, sister chromatid exchanges and gene mutations in human cells *in vitro*. Micronucleus (MN) is a nuclear body originated by chromosome breakage or chromosome segregation during cell division. It is derived from chromosomal fragments and whole chromosome lagging behind in anaphase. The two basic phenomena leading to the formation of MN in mitotic cells are chromosome breakage and dysfunction of the mitotic apparatus. MN is widely used as biomarkers for genotoxicity.[4]

The present study highlights the effects of FA in workers in Anatomy Departments. Frequency of MN has been compared with that of the comparison group. As there are very limited studies on FA exposure in India, the present study has been undertaken to detect the genotoxic effect of formaldehyde with respect to the duration of exposure. By knowing the damage caused by FA it is easy to reduce the occupational exposure by using appropriate protective measures.

METHODS

The present cross sectional study was carried out in the Division of Human Genetics, St. John's Medical College, Bangalore. It was conducted in 2012. The sample size was calculated using N-master software. Ethical clearance was obtained from the Institutional Ethics Review Board (IERB).

Thirty male workers in Anatomy labs, having exposure to FA during embalming and handling the specimens in various medical colleges were selected for the study after taking an informed consent. Thirty people, not exposed to FA at any point of time were taken as comparison group. An appropriate questionnaire was used to collect the information about the duration of exposure and usage of protective measures. People with habit of smoking, history of long term medical illness, cancer and treatment, exposed to frequent X- Rays and other kinds of radiation were excluded from the study to avoid the confounding effect. As there were no female workers involved with FA exposure, females were excluded both from study and comparison groups.

Protocol

Buccal cells were scraped from the inner aspect of cheek after rinsing the mouth thoroughly. The cells were transferred to the centrifuge tubes containing 5ml of 0.9% saline and were centrifuged twice. Supernatant was discarded and 2 drops of fixative (3:1 Methanol and Acetic acid) was added. Using Pasteur pipette, the cells were dropped on chilled slide and air dried. Two slides were prepared per sample. The slides were fixed in fixative for 20 minutes, air dried, labelled and stored for 24 hours before staining. The slides were stained using 1:6 Giemsa solution. The MN were identified according to the criteria given by Fenech M.[5] Figure 1 shows the buccal cell with micronucleus. The investigator was blinded about the details of study population. The frequency of MN per 1000 cells was taken as the variable for statistical analysis.

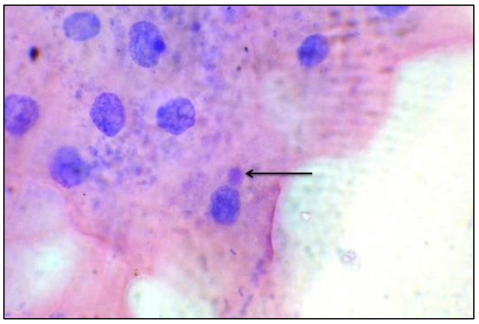

Figure 1: Buccal cell showing micronucleus (Giemsa stain, 40 X magnification).

SPSS version 16 was used for the statistical analysis. Student-t test and Pearson's correlation were used for the analysis.

RESULTS

The study included 30 male workers in the anatomy departments of various medical colleges who were exposed to FA during routine handling of specimens and embalming, and 30 people not exposed to FA as the comparison group. The general characteristics of the study population are described in Table 1.

Table 1: General characteristics of the study population.

		Exposed	Comparison group
Age (in years)	N	30	30
	Mean and SD	39.9 ± 1.49	37.8 ± 1.06
	Range	24 - 55	18 - 56
Years of exposure	Mean	10.66	
	Range	1 - 30	

The mean and standard deviation (SD) of buccal cells in exposed was 15.13 ± 4.77 and in controls was 5 ± 3.06. There was a significant increase in the frequency of MN in exposed group when compared with the comparison group ($p<0.001$).

The maximum number of exposed had a MN range between 11- 15 and the comparison group it was between 6- 10 (Figure 2).

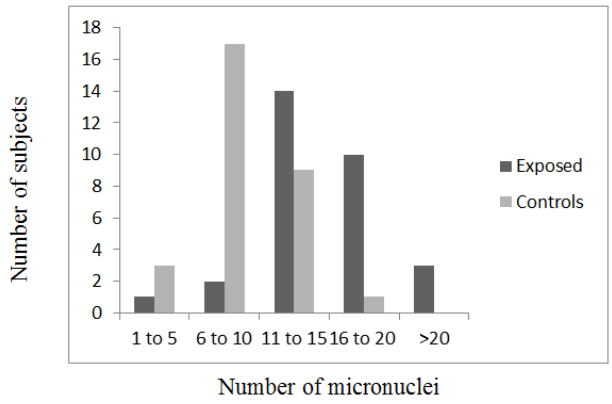

Figure 2: Frequency distribution of MN in exposed and controls. Maximum number of exposed have a MN range between 11 - 15 and controls have 6 - 10.

Pearson's correlation test was used to correlate between the duration of exposure and frequency of MN. There was a positive correlation between the years of FA exposure and the number of buccal cell MN indicating that DNA damage due to FA was directly proportional to the duration of exposure (r=0.5, p=0.03) (Figure 3).

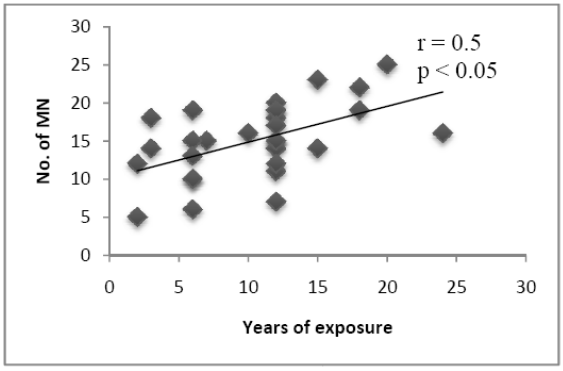

Figure 3: Scatter diagram showing a positive correlation between the years of exposure and number of MN in buccal cells.

Age of the person did not seem to have significant effect of MN formation (r=0.2, p=0.08).

The effectiveness of protective measures was not assessed as the workers were not using them regularly.

DISCUSSION

Formaldehyde is one of the commonly used chemical for preservation of cadavers and dissected specimens in Anatomy labs the people working are exposed to a relatively high concentration. The literature shows number of studies on the adverse genetic effects of formaldehyde. Buccal cell MN Assay is one of the commonly used methods for analysing the DNA damage.

Takahashi et al. evaluated the health effects of FA on medical students in which physical symptoms, including skin irritation, eye soreness, lacrimation, rhinorrhea and throat irritation were the commonest complaints[4]. Numerous other examples of chronic health effects from long-term/low dosage exposure scenarios are reported in anatomy assistants, autopsy pathologists, dissectors, morgue workers and wood plant workers.

A study by Wunnapuk et al. revealed a significant increase in MN in buccal cells in dentistry students after exposure to FA during Anatomy course.[6]The present study also showed a significant increase in the frequency of MN in buccal cells.

Viegas et al. studied a population exposed to high peak concentrations of formaldehyde with a long-term exposure on buccal cells and peripheral lymphocytes. They report that these two factors cumulatively, can be the cause of the observed genotoxic endpoint effects. The association of these cytogenetic effects with formaldehyde exposure gives important information to risk assessment process and may also be used to assess health risks for exposed workers. The genotoxic effects of FA are directly proportional to the duration of the exposure. The study by Viegas et al. showed a positive correlation between the duration and MN frequency.[7] Present study highlights that the DNA damage is directly proportional to the duration of exposure to FA.

The literature also shows number of studies like Viegas et al., Gabriel et al. found no significant association between smoking and MN formation.[7,8] But there are few studies like Hans et al in which a significant association was found.[9]

The studies done on buccal cells and lymphocytes show a positive correlation between age and number of MN indicating increased DNA damage with advanced age. A study on Greek farmers by Pastor et al revealed a positive correlation between age and number of MN.[10] The present study showed a weak positive but not significant correlation between the two parameters.

Failure to measure the levels of FA was the drawback of the study. As the workers were not using the appropriate protective measures, their effectiveness could not be assessed.

CONCLUSION

The present study was done to assess the DNA damage in people who were exposed to FA and a comparison group not exposed to FA by buccal cell Micronucleus Assay. There was a significant increase in the buccal cell MN in people exposed to FA which was directly proportional to the duration of exposure.

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Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics review board

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