

**Original article:**

## Micronuclei assay in buccal smear of formalin exposed individuals

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### ABSTRACT:

**Background:** Formalin is considered to be genotoxic contaminant. Prolonged exposure to formalin may lead to formation of micronuclei and other nuclear abnormalities.

**Aim:** To detect the presence of micronuclei in buccal smear of formalin exposed individuals. Teaching staff, lab personnel and students form the target group to be exposed to formalin in the dissection lab or embalming room or museum of the anatomy department. Formaldehyde exhibits the genotoxicity by forming DNA protein cross links and micronuclei. The present study is done to demonstrate the micronuclei in persons exposed to formalin for varying periods of time.

**Materials & methods:** The study group (n=50) comprised of teaching faculty & lab personnel from the department of Anatomy and students of 1<sup>st</sup> BDS & 2<sup>nd</sup> MBBS. They were categorised into 5 groups based on the duration of exposure to formalin. Buccal smears were made from the buccal scrapings collected by a wooden spatula. The smears were stained with methylene blue and observed under light microscope for the presence of micronuclei.

**Results:** The number of micronuclei increased with increasing duration of exposure to formalin. The mean number of micronuclei observed in the buccal smears of formalin exposed to 10-15 years was 2.66 while it was 0.33 in those exposed to less than a year. The mean values correlated with other studies.

**Conclusion:** Micronuclei have been detected in the buccal smears of individuals exposed to formalin. The number of micronuclei has been increasing with the years of exposure to formalin. Hence continuous exposure to formalin can be considered as an occupational health hazard.

**KEYWORDS:** Micronucleus, formalin, exposure, buccal smear, genotoxicity.

### INTRODUCTION

Formalin is a dissolved gas of Formaldehyde in concentrations from 37% to 54% <sup>[1]</sup>. Continuous exposure to formalin is hazardous to health. Formalin is used extensively in the department of Anatomy at dissection hall, museum and in the embalming room. The pungent smell of formalin causes a variety of adverse effects like skin rashes, headache and sinusitis. Formalin exhibits its genotoxicity by forming DNA-protein crosslinks<sup>[2]</sup>. The genotoxic effect of formalin is reflected in the form of micronucleus or double nucleus. A micronucleus is the isolated part of the nucleus which contain acentric or whole chromosomes that are unable to join the mitotic spindles during cell division. The buccal micronucleus assay in exfoliated buccal cells is utilized as biomarker for DNA damage, cell death and basal cell frequency<sup>[4]</sup>. Occupational exposure for formaldehyde is 1 ppm 8-hour time-weighted average (TWA) and 2 ppm for short-term exposure limit (STEL). Also, the national cancer institute has included formaldehyde as a carcinogen<sup>[3]</sup>. Teaching staff, lab personnel and students form the target group to be exposed to formalin in the dissection lab or embalming room or museum of the Anatomy department. The present study is designed to detect the presence of micronuclei in buccal smear of formalin exposed individuals.

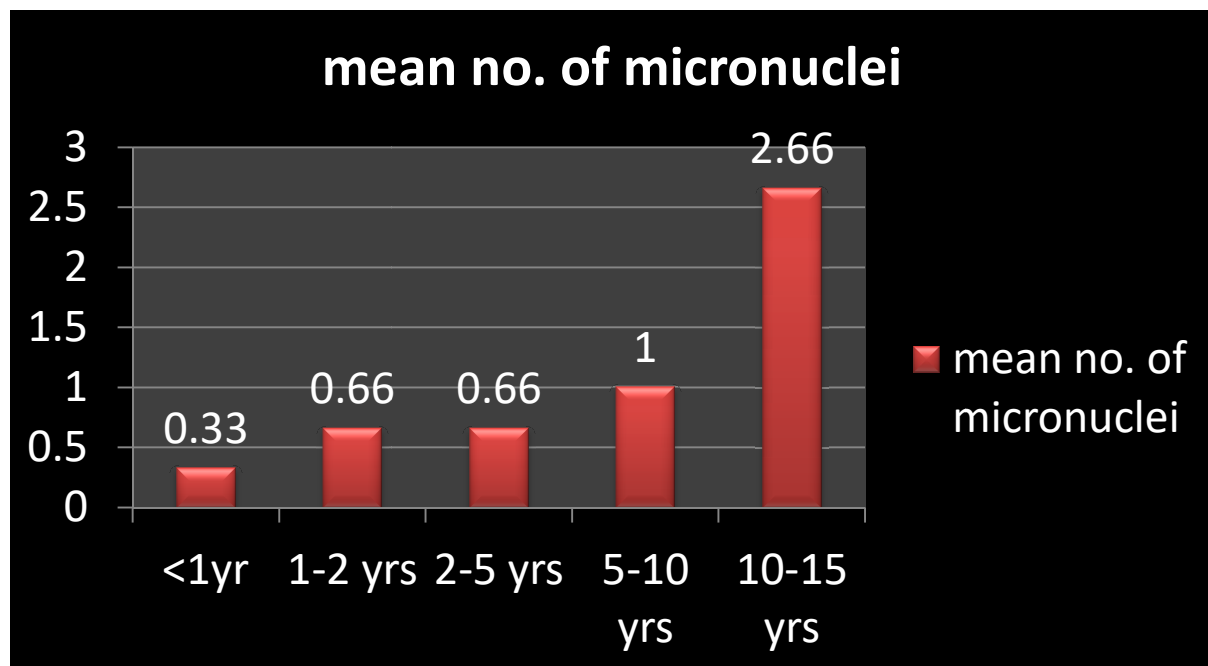
## MATERIALS & METHODS

The study group (n=50) comprised of teaching faculty & lab personnel from the department of Anatomy and students of 1<sup>st</sup> BDS & 2<sup>nd</sup> MBBS. They were categorised into 5 groups based on the duration of exposure to formalin. Buccal smears were made from the buccal scrapings collected by a wooden spatula. The smears were dried and stained with methylene blue. Each slide is observed under light microscope for the presence of micronuclei in 10x, 40x and 100x magnification. 300 cells from each slide were studied. Few cells without nucleus were not counted. Each buccal smear is prepared with utmost care to obtain single cell thickness and to avoid clumping of the squamous cells. Though other staining procedures like H&E, Giemsa and Papanicolou stains were available, methylene blue staining technique is done as it is considered to be the most reliable and the simplest procedure to visualise the micronuclei.

## RESULTS

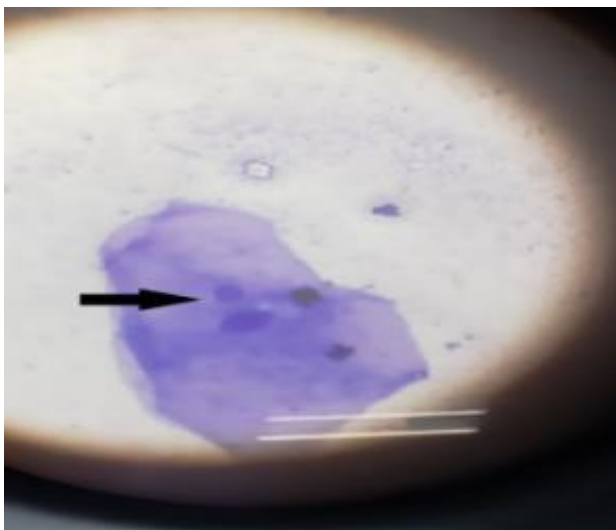
The following graph depicts the ratio between mean number of micronuclei and years of exposure. The graph shows an increasing pattern with prolonged years of exposure.

Graph 1: mean number of micronuclei versus years of exposure to formalin

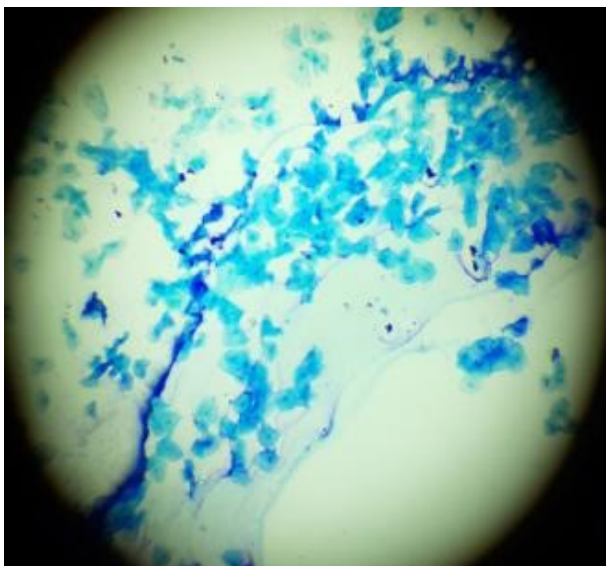


The following slide illustrates single micronuclei in a single squamous epithelial cell of the buccal smear under 100 x magnifications. The micronucleus can be seen as a separate mass more or less rounded and with the same intensity of staining as the main nucleus and is situated close to it. The other two masses to the right side of the cell are artefacts as their staining is different from the main nucleus and also they are not circular.

**Slide 1: methylene blue staining of buccal smear under 100x showing a micronucleus in a single buccal squamous epithelial cell.**



**Slide 2: buccal smear with methylene blue staining under 40x magnification**



**Table 1: comparison of results of present study with others.**

S No.	Study by	Range of mean number of micronuclei
1.	VEIGAS etal	0.64 ± 1.74
2.	SHEKHAWAT etal	0.8 to 9.6
3.	Present study	0.33 to 2.66

**DISCUSSION**

Micronucleus in a buccal smear is a marker for genotoxic contamination. Commonly they are formed during anaphase from lagging acentric chromosome or chromatid fragments. This may be due to hypomethylation of repeat sequences present in pericentromeric DNA or irregularities in kinetochore protein or flawed anaphase checkpoint genes. Studies<sup>[6,7]</sup> show the carcinogenic effect of formalin on upper respiratory tract. A study<sup>[8]</sup> concludes that low level exposure to formaldehyde is associated with cytogenetic changes in the buccal

epithelial cells and blood lymphocytes. Neuss<sup>[9]</sup> et al determined that formaldehyde remains in the cell culture medium for a longer time despite its high reactivity with macromolecules. The prevalence of micronuclei has been proved to increase with increasing duration of formalin exposure. The present study determines the micronuclei in a considerable number of buccal smear slides prepared with methylene blue staining. The results of the present study correlates with the values of previous studies<sup>[10,11]</sup>.

## CONCLUSION

Increased number of micronuclei has been detected in the individuals exposed to formalin for longer duration. Counselling has been given and advised to reduce their exposure to formalin.

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