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Original Research Article

Effect of Years of Exposure on Micronucleus Frequency in Buccle Epithelium Cell through Micronucleus Assay in Subjects Exposed to Formaldehyde

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Abstract

Background: The stratified squamous cell epithelium continuously loses superficial buccal cells, which are replaced by cell division. When these cells split, chromosomal fragments or full chromosomes may be left behind during mitotic anaphase and manifest as tiny nuclear particles, or micronuclei, in the cytoplasm of daughter cells. The cytogenetic changes can only be examined from the exfoliated cells after the cells have matured and migrated to the surface. A novel genotoxicity method that shows promise for the investigation of epithelial carcinogens is the micronuclei assay (MA) on exfoliated buccal cells. For detecting tissue-specific genotoxic damage in people exposed to carcinogenic combinations, micronuclei (MN) are ideal internal dosimeters.

Aim: To analyse effect of years of exposure on micronucleus frequency in buccle epithelium cell through micronucleus assay in subjects exposed to formaldehyde.

Methods and Materials: After obtaining informed consent, 50 male and female participants who are exposed to formaldehyde were chosen for the study. The duration of formaldehyde exposure in days, months, and years was recorded. After properly washing the mouth, buccal cells were scraped off the inside of the cheek. The cells were moved into centrifuge tubes with 5ml of 0.9% saline, and they underwent two centrifugal separations. 2 drops of fixative (3:1 Methanol and acetic acid) and the supernatant trash were added. The cells were dropped onto a cooled slide using a Pasteur pipette before being air dried. Per sample, two slides were created. The slide was air dried, labelled, and fixed in fixative for 20 minutes before being stored for 24 hours before staining. Giemsa staining solution was used to mark the slides. The criteria provided by Tolbert *et al* that were used to identify the MN.

Results: There was positive correlation of various parameters of degree of exposure to formaldehyde with MN count /500 cells but statistically significant correlation was with year of exposure and total exposure only. ($p=0.004$ & $p=0.003$ respectively).

Conclusion: It was concluded that years of exposure to formaldehyde were significantly correlated to the frequency of micronucleus. Micronucleus assay in human epithelial buccal cells is most reliable and attainable method to assess occupational menace resulting in genetic abnormality due to environmental and chemical factors.

Keywords: Micronuclei, Microassay, Formaldehyde, Years Of Exposure.

Introduction

Achromatic gas with a strong smell is formaldehyde (CH2O). Aleksandr Butlerov first produced formaldehyde in 1859, but it was August Wilhelm von Hofmann who determined how it is formed. Formaldehyde is the most basic yet volatile of all aldehydes. In 1867, he found that the oxidation of methanol into formaldehyde occurs when it is passed through a heated platinum spiral. This is the basis for modern industrial synthesis of formaldehyde, which involves oxidising methanol using a metal catalyst. The early 20th century saw the emergence of a new material called plastics as a result of advances in chemistry and physics, as well as consumer desires for more inventive synthetic goods [1,2].

It is a frequently used preservative in mortuaries and medical labs. Formaldehyde exposure at work is associated with a number of risks, including allergic reactions and genetic harm. One of the most frequent exposure pathways is through inhalation, particularly in jobs that expose workers to formaldehyde vapour, such as those in anatomical and pathological laboratories. Conjunctival and mucosal irritation, especially in the respiratory system, occupational asthma, occupational dermatitis, and other allergic reactions are all brought on by formaldehyde [3,4]. Chemicals, wooden objects, glues, permanent press textiles, paper product coatings, and fiberboard are further items that contain formaldehyde. It is a typical industrial disinfectant, fungicide, and bactericide. The synthesis of formaldehyde in the troposphere is caused by the oxidation of hydrocarbons. Low amounts of this gas are created in the environment as an intermediary in the methane cycle. It is one of the volatile molecules created during the initial stages of soil-borne plant wastes decomposition [5-15].

The nuclear body that results from chromosomal segregation or breaking during the mitosis and meiosis kinds of cell division is known as a micronucleus (MN) New body cells are created via the process of mitosis, a form of cell division that results in two daughter cells with the same number and type of chromosomes as the parent nucleus, while meiosis is a type of cell division that produces four daughters with half as many chromosomes as the parent cell each. The type of cellular division known as meiosis is what creates egg and sperm cells. A vital process for life is mitosis [7-17].

The stratified squamous cell epithelium continuously loses superficial buccal cells, which are replaced by cell division. When these cells split, chromosomal fragments or full chromosomes may be left behind during mitotic anaphase and manifest as tiny nuclear particles, or micronuclei, in the cytoplasm of daughter cells. The cytogenetic changes can only be examined from the exfoliated cells after the cells have matured and migrated to the surface.

An novel genotoxicity method that shows promise for the investigation of epithelial carcinogens is the micronuclei assay (MA) on exfoliated buccal cells. For detecting tissue-specific genotoxic damage in people exposed to carcinogenic combinations, micronuclei are ideal internal dosimeters. Through a micronucleus assay, this study examined how exposure to formaldehyde over time affected the frequency of micronuclei in buccal epithelial cells [9-11].

This study was conducted to analyse effect of years of exposure on micronucleus frequency in buccle epithelium cell through

micronucleus assay in subjects exposed to formaldehyde

Methods and Materials

After obtaining informed consent, 50 male and female participants who are exposed to formaldehyde were chosen for the study. The duration of formaldehyde exposure in days, months, and years was recorded. 50 individuals who had not been exposed to formaldehyde served as controls. An appropriate questionnaire was used to assess the general health conditions, medical history, long-term medications, radiation exposure, lifestyle factors, including habits and addictions, and specific protective measures, such as the use of masks, gloves, and aprons while working with formaldehyde.

Inclusion criteria:

1. Subjects who were exposed to formaldehyde for at least one year and up to thirty years
2. Control subjects who had never been exposed to formaldehyde.
3. Participants willing to engage in the study who are at least 18 years old.

Exclusion criteria:

1. Those who have received cancer therapy in the past.
2. Individuals who frequently receive X-rays and other types of radiation.
3. Both the research and the control groups exclude anyone who works in the paint or pesticide industries, which are known to be carcinogens.
4. Individuals who are unwilling to take part in the study.

Method of sample collection

After properly washing the mouth, buccal cells were scraped off the inside of the cheek. The cells were moved into centrifuge tubes with 5ml of 0.9% saline, and they underwent two centrifugal separations. 2 drops of fixative (3:1 Methanol and acetic acid) and the supernatant trash were added. The cells were dropped onto a cooled slide using a Pasteur pipette before being air dried. Per sample, two slides were created. The slide was air dried, labelled, and fixed in fixative for 20 minutes before being stored for 24 hours before staining. Giemsa staining solution was used to mark the slides. The criteria provided by Tolbert *et al* [12] that were used to identify the MN have the following criteria for scoring:

1. The MN ought to be smooth and rounded.
2. The MN's diameter should be about one-third of the associated nucleus.
3. The MN should be bright and dark in the lighting field.
4. The MN and nucleus should have comparable staining intensities.
5. The MN texture ought to resemble that of a nucleus.
6. It need to have the same focal plane as the nucleus.
7. No bridge or overlap with the nucleus.

Instruments

Giemsa solution, 100% alcohol, xylene, centrifuge tubes, saline, fixative (3:1 methanol and acetic acid), Pasteur pipette.

Statistical Analysis

Using a step-by-step methodology, multivariate regression analysis of several independent factors for MN count/500 cells was performed. The likelihood of leaving the model was maintained at >0.10, whereas the probability of remaining in the modal was maintained at <0.05. Age, sex, smoking and drinking histories, nutrition, family history of diabetes, hypertension, and formaldehyde exposure were all entered into the model. Age and formaldehyde exposure were the only independent variables left in the model. R2, or the model's coefficient of determination, was 0.6471. The model's substantial

prediction was found by the analysis of variance. ($p<0.001$)

Regression equation obtained was as follow-

Mn count/500 cells= - 1.2749+0.1101(age) + 6.3688(exposure to formaldehyde)

Results

Male participants were 68% in case and 48% in control groups respectively.

On application of Fisher Exact test, both groups were found comparable w.r.t. gender distribution. ($p= 0.068$). (Table 1) There was positive correlation of various parameters of degree of exposure to formaldehyde with MN count /500 cells but statistically significant correlation was with year of exposure and total exposure only. ($p=0.004$ & $p=0.003$ respectively). (Table 2)

Table 1: Distribution of study participants according to gender in case and control group

Gender	Group				Total	
	Case		Control			
	No.	%	No.	%	No.	%
Male	34	68.00	24	48.00	58	58.00
Female	16	32.00	26	52.00	42	42.00
Total	50	100.00	50	100.00	100	100.00

Fisher Exact Test P = 0.068

Table 2: Correlation of degree of exposure of formaldehyde with MN count/500

Parameters	N	Coefficient correlation (r)	P value	95% CI for r
Number of hours in a day	50	0.1641	0.2549	-0.1198 to 0.4231
Number of days in a week	50	0.08924	0.5377	-0.1939 to 0.3587
Years of exposure	50	0.4791	0.0004	0.2316 to 0.6683
Total exposure	50	0.4889	0.0003	0.2438 to 0.6754

Discussion

Superficial buccal cells are shed continuously and are replaced by cellular division in the stratified squamous cell epithelium. When these cells divide, chromosome fragment or whole chromosome can be left behind during mitotic anaphase and appear in the cytoplasm of daughter cells as small nuclear particles, called micronuclei. After the cells mature and migrate to the surface, the cytogenetic changes ban be analysed from the exfoliated cells. As the exfoliated buccal cells provide an accurate visualization of MN, these cells are commonly used. The micronuclei assay (MA) in exfoliated buccal cells is an innovative genotoxicity technique, which holds promise for the study of epithelial carcinogens [12,13] Micronuclei are suitable internal dosimeters for revealing tissue-specific genotoxic damage in individuals exposed to carcinogenic mixtures. This study was conducted to analyse effect of years of exposure on micronucleus frequency in buccle epithelium cell through micronucleus assay in subjects exposed to formaldehyde.

In the current investigation, cases were divided into 3 groups based on the level of exposure. Only a positive connection (P=0.004,.003) between years of exposure and total exposure was discovered. A 50-person study was undertaken by Shekhawat *et al* (25 control or non-exposed subjects, 25 Subjects exposed to formalin vapours). They came to the conclusion that the frequency of micronuclei similar to those in our study was substantially linked with the number of years

of formaldehyde exposure. (p=0.0000) [18] Similar to this, Veigas *et al*. claimed that there is still a positive link between the frequency of micronuclei in epithelial cells and the length of formaldehyde exposure. (91) ($p < 0.01$) [13].

When a chromosome or chromosome fragment isn't incorporated into one of the daughter nuclei during cellular division, a small nucleus known as a micronucleus arises. It often serves as a sign of chromosomal mutability and genotoxic events. The frequent presence of micronuclei in malignant cells indicates genetic damaging events that can raise the likelihood of developing or progressing degenerative illnesses. During anaphase, micronuclei form from lagging acentric chromosome or chromatid fragments brought on by improperly or incompletely repaired DNA breaks or by chromosome nondisjunction. This misaligned chromosome segregation can be brought on by defective anaphase checkpoint genes, deformed kinetochore proteins, duplicate sequences in the pericentromeric DNA, faulty spindle apparatus, or hypomethylation of these sequences [19,20].

The micronucleus test using exfoliated cells has many advantages because it is a simple and fast test system. Tolbert *et al* [12] have provided the following criteria for scoring, which will be used to determine the MN. The MN should be smooth and rounded, and its diameter should be approximately one-third of the associated nucleus. The MN should be both bright and dark in the illumination field. The nucleus and MN should have comparable staining intensities. The nucleus-like texture of the MN should be used. It ought to be in the same focal plane as the nucleus. No bridge or overlap with the nucleus.

Wunnapuk K *et al* evaluated the MN in buccal cells in students exposed to embalming solution vapour and stained with Feulgen plus Fast Green. The results showed a significant increase in the total number of micronuclei in mononucleated buccal cells [20] Titenko N *et al* evaluated the epithelial cell micronuclei by fluorescence in situ hybridization (FISH) in mortuary science students exposed to formaldehyde. He noted a significant increase in the frequency of MN and also explained that, the primary mechanism of micronucleus formation is chromosomal breakage [21]. Nersesyan AK *et al* reviewed about 20 articles on MN assay and also the shortcomings of several of them are discussed. These results are compared whenever possible with literature data. The aim of this mini – review can be a criticism of the shortcomings of these papers so as to stimulate improvement of the presentation of micronucleus assay data, which might allow the comparison of these results with data presented by foreign investigators [22].

The formaldehyde is so annoying to the nasal passages of humans at levels that produce tumours in laboratory animals and humans simply can't tolerate it even for a few minutes. All of the available and emerging human health research data shows that if formaldehyde exposure is kept below levels that produce chronic irritation and visible target tissue damage, the risk of cancer is essentially zero. A study on medical students' exposure to formaldehyde in a gross anatomy dissection by Lakchayapakorn K *et al* studied that the mean of formaldehyde concentrations in the breathing zone of medical students and instructors was significantly higher than the mean of eyes and burning nose [23]. They conclude by recommending that even though formaldehyde concentrations were relatively low, medical students, instructors and cadaver-related workers should wear personal protective devices to reduce the effect of gaseous formaldehyde exposure during gross anatomy laboratory of cadaver

contact. A study done on the recommended occupational exposure limit for formaldehyde based on irritation by Paustenbach D *et al* studied that, any occupational or environmental guideline for formaldehyde should be based totally on controlled studies in humans, since nearly all other studies are compromised by the presence of other contaminants. They concluded that if concentrations of formaldehyde are kept below 0.1 ppm in the indoor environment (where exposure might occur 24h/d) this should prevent irritation in virtually all persons [24].

The results indicated above, combined with the most recent effects of human carcinogenicity, point to the necessity of meticulous FA exposure surveillance. Controlling the risk associated with occupational exposure to FA requires careful planning of training and medical surveillance programmes as well as the execution of preventative measures.

Conclusion

It was concluded that years of exposure to formaldehyde were significantly correlated to the frequency of micronucleus. Micronucleus assay is most reliable and attainable method to assess occupational menace resulting in genetic abnormality due to environmental and chemical factors. Micronucleus assay in human epithelial buccal cells is most conventional and widely used method for assessing impact of nutrition lifestyle habits such as smoking drinking alcohol, genetic and cytotoxic exposure, risk of accelerated ageing, and neurodegenerative diseases.

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