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Original Article

Cytogenetic Effects of Exposure to Formalin Vapours in Buccal and Nasal Mucosae: A Study in the Students of Anatomy and Embalmers

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ABSTRACT

Presently formaldehyde has been classified as group I carcinogen to the human population by International agency for research on cancer, as its exposure has been associated with the nasal, nasopharyngeal, buccal carcinoma and leukemia. Formaldehyde on reaching DNA forms DNA-Protein crosslinks thereby causing chromosomal mutations and micronuclei formation. The micronucleus assay provides information of cytogenetic damage in tissues by these carcinogens. In present study the samples from buccal and nasal mucosae of 25 exposed and 25 non-exposed individuals were taken with the help of wooden spatula and cytopathology brush respectively. These samples were immediately smeared on the microscopic slides and fixed with ethyl alcohol and then stained with the Papanicolaou stain. After staining, slides were studied by the light microscope under 400X and 1000X magnification for the micronuclei and other effects causing genocytotoxicity. Our results show a significant increase in Micronuclei and Micronucleated cells frequency in exposed groups compared with the controls. A significant positive correlation between micronuclei frequencies and duration of exposure is found(r=0.7332 and p Value=0.00).

KEYWORDS: Buccal mucosa, Genotoxicity, Micronucleus, Formalin vapours.

INTRODUCTION

Formaldehyde (HCHO), the most simple and reactive of all aldehydes, is a colorless, and readily polymerizing gas at room temperature[1]. It has a pungent suffocating odor that is recognized by most human subjects at concentration below 1ppm[2]. HCHO was discovered by Butlerov in 1859. HCHO is commercially sold as formalin, a methanol-stabilized water solution containing 37, 44 or 50% formaldehyde.

Formaldehyde is a sensitizing agent that can cause an immune system response upon initial exposure. Acute exposure is highly irritating to the eyes, nose & throat and may even produce severe allergic reactions of the skin, eyes and respiratory tract. Ingestion of Formaldehyde can be fatal. Long term exposure to low level of Formaldehyde cause asthma like respiratory problems and skin irritation such as dermatitis and itching[3]. According to code of Federal Regulations (CFR), the permissible exposure limits (PFLs) of Formaldehyde in the workspace covered by the standard are 0.75ppm[4]. It is present at level of between 0.12 and 0.39 ppm in our atmosphere. Presently, formaldehyde has been classified as group I carcinogen to the human population by International agency for research on nasal, nasopharyngeal, buccal carcinoma and leukemia[5]. Formaldehyde on reaching DNA form DNA-Protein crosses links thereby causing chromosomal mutations and micronuclei formation[6]. Micronuclei test provides a reliable measure of chromosomal breakage and loss at lower cost and more easily than chromosomal aberrations.

The micronucleus assay provides information of cytogenetic damage in tissues by these carcinogens. Therefore this study has been undertaken as it can be used as a valuable screening tool and risk assessor for carcinoma of the buccal and nasal mucosae in the population exposed to formalin. Baseline frequencies for micronucleated cells in the BM are usually within the 0.5 -2.5 micronuclei/1000 cells range. Exfoliated cell micronucleus assay also demonstrates certain background prevalence's nuclear anomalies.

The following nuclear abnormalities can be observed in exfoliated smears:-

1. Binucleation – Binucleated cells are cells containing two main nuclei instead of one. They are probably indicative of failed cytokinesis following the last nuclear division in the basal cell layer.

2. Pyknosis or shrunken nuclei- Pyknosis is a stage in programmed cell death in which the chromatin in the nucleus of a cell polymerizes and condenses into a solid structure less mass.

3.Karyorrhexis or nuclear disintegration: Involving loss of the nucleus. These cells may be undergoing a late stage of apoptosis, but this has not been conclusively proven.

4. Karyolysis or nuclear dissolution- Karyolitic cells are cells in which the nucleus is completely depleted of DNA and thus apparent as a ghost-like image. These cells represent a very late stage in the cell death process.

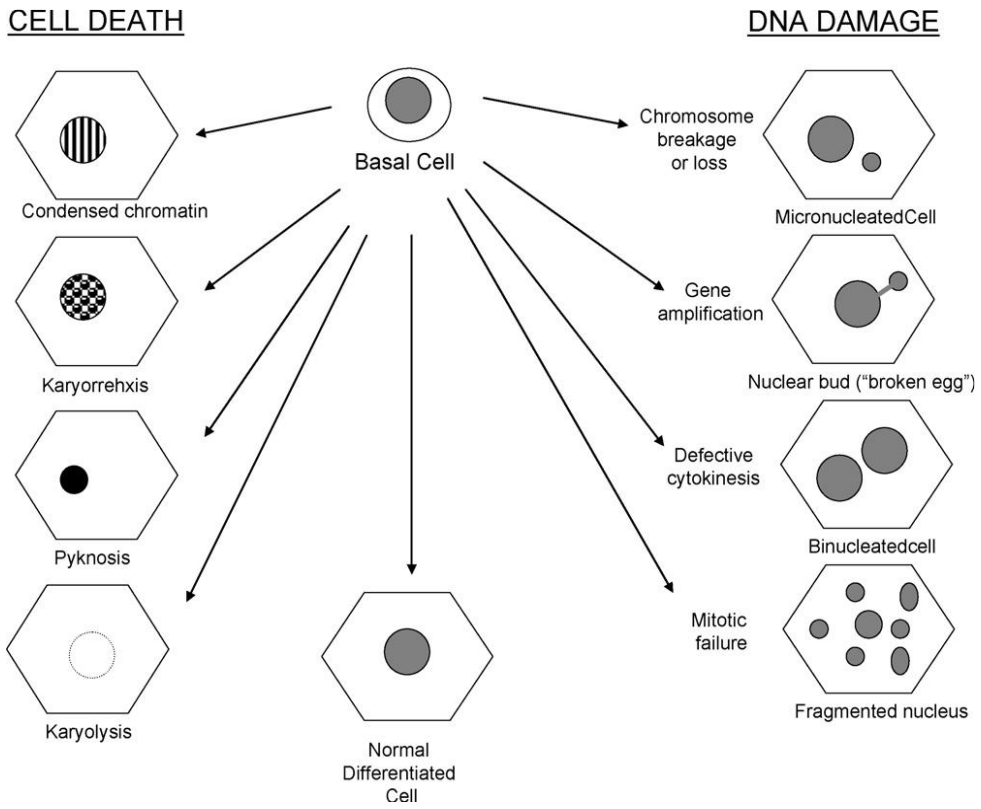

Fig 1: Buccal MN assay cytome model. Schematic diagram of different types of buccal cells and the possible mechanism for their origin.

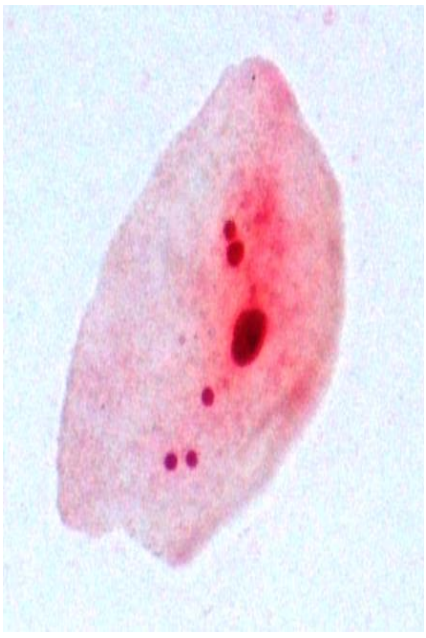

Fig 2: Micronucleated cell with five micronuclei

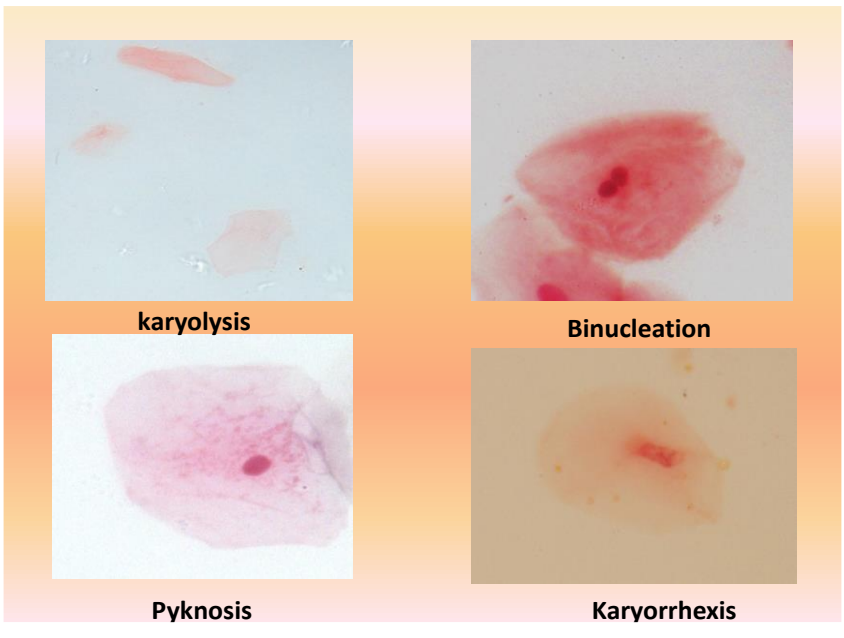

Fig 3: Various Nuclear Abnormalities

MATERIALS & METHODS

For this study, total 50 subjects (25 control or non-exposed subjects, 25 Subjects exposed to formalin vapours) were selected. The age ranged between 17-60 years. Minimum duration of exposure for cases was 4 months. Attention was paid to exclude any individuals who had been subjected to any X-ray treatment or antibiotic therapy 3 weeks prior to when samples were taken. Individuals having no history of alcohol use, smoking, tobacco chewing in any form were selected. Persons having any benign, premalignant lesion and carcinoma of nose & oral cavity and any deformity of nose like DNS etc. were excluded.

Collection of samples and staining:-

Subjects were asked to rinse their mouth with water and a pre-moistened wooden spatula was used for collection of sample from buccal mucosa. Similarly, sample was taken with cytopathology brush from the mucosa of the inferior turbinate of the nose. The sample was immediately smeared on the microscopic slides and fixed with ethyl alcohol. Stain used in this study was Papanicolaou stain. The stained slides were studied under 400X magnification of research microscope. A total of 1000 cells with well-preserved cytoplasm were examined for each subject. From each slide, the cells recorded were the total number of normal cells and of micronucleated, pyknotic, karyorrhectic, karyolitic and binucleated cells. This information was then used to calculate the frequency of the various cell types.

Criteria suggested by Tolbert et al.[7] :-

a. For the cells to be scored in study :-

Intact cytoplasm and relatively flat cell position on the slide.
Little or no overlap with adjacent cells.
Little or no debris.
Nucleus normal and intact, nuclear perimeter smooth and distinct.

b. For the identification of micronuclei in cells :-

Rounded smooth perimeter suggestive of a membrane.
Less than a third the diameter of the associated nucleus but large enough to discern shape and colour.
Staining intensity similar to that of the nucleus.
Texture similar to that of nucleus.
Same focal plane as nucleus.
Absence of overlap with or bridge to the nucleus.

		n	MEAN	S.D.	p Value
Buccal	case	25	0.365	0.154	<0.05 (o.oo)
	control	25	0.0734	0.018	
nasal	Case	25	0.170	0.033	<0.05
	control	25	0.121	0.042	

Table-1: Micronucleus frequency in exfoliated buccal cells.

	n	MEAN	S.D.	p Value
Buccal	25	0.365	0.154	<0.05
nasal	25	0.170	0.033	

Table-2 : Comparison between nasal & buccal micronuclei/1000 cells

Duration of exposure	n	mean	S.D.	p Value
<1 yr	17	0.308	0.087	0.00
1-5 yrs	6	0.400	0.096	
>5 yrs	2	0.741	0.230	

Table-3: Relation with duration of exposure & micronuclei/1000 cells in buccal mucosa

Duration of exposure	n	Mean	S.D.	p Value
<1 yr	17	0.164	0.034	0.231
1-5 yr	6	0.176	0.020	
>5 yr	2	0.206	0.051	

Table -4: Relation of duration of exposure & Micronuclei /1000 cells in nasal mucosa

OBSERVATIONS

Formaldehyde induces various kinds of DNA damage. Current predictive indicators of DNA damage are chromosomal aberrations and micronuclei. A micronucleus is a small extra nucleus separated from the main nucleus and is generated during cellular division when a chromosome fragments or divides late. Micronucleus assay has been used to determine the genotoxic and mutagenic potentials of various physical and chemical agents which could lead to the production of micronuclei.

Table 1 present average frequency of Micronuclei in buccal cells with pap staining is 0.365±0.154 in cases and 0.0734 ±0.018 in control, however, this difference is statistically significant. From the present study, the gradient of MN frequencies in buccal region of cases is significant in comparison to control. The p Value is <0.05.Table 2 reveals comparison between nasal & buccal micronuclei/1000 cells**.** The increase in nasal epithelial micronucleus frequencies was small compared that in buccal cells.

Table 3 & 4 reveal relation with duration of exposure & micronuclei/1000 cells in buccal mucosa nasal mucosa respectively. A significant result was found between duration of exposure to formaldehyde (year of exposure) and frequency of micronuclei in the epithelial cells (<0.05). This indicate that, exposure duration also has relevance for the development of health effects, similarly, a significant result was found between duration of exposure to formaldehyde (year of exposure) and frequency of micronuclei in the nasal epithelial cells (<0.05) but less than as compared to buccal mucosa.

		BUCCAL		NASAL	
		mean	S.D.	mean	S.D.
Pyknosis	Case	0.005	0.002	0.004	0.002
	Control	0.001	0.001	0.001	0.0009
binucleation	Case	0.006	0.003	0.004	0.002
	Control	0.002	0.001	0.002	0.001
Karyorrhexis	Case	0.006	0.003	0.004	0.001
	Control	0.001	0.001	0.001	0.0009
karyolysis	Case	0.014	0.005	0.006	0.003
	control	0.0046	0.001	0.003	0.001

Table-5: Cytoxic changes in Buccal and Nasal mucosae.

Study	Buccal micronuclei/1000 cells	Nasal micronuclei/1000 cells
Susana Viegas et al.[8]	0.64±1.74 p Value-0.031	-------
Suruda et al.[9]	0.60±1.27 p Value-0.25	0.50±0.67 p Value-0.33
Titenko-Holland N et al.[10]	2.0±2.0 p Value-0.007	2.5±1.3 p Value-0.20
Wunnapuk et al.[11]	2.53±1.72	-------
Holland et al.[12]	0.33	---
Carina ladeira[13]	0.96±0.277	---
Present study	0.365±0.154 p Value-0.000001	0.170±0.033 p Value-0.000001

Table 6: Comparison of Different studies.

Table5 reveal Cytoxic changes in Buccal and Nasal mucosae. Along with micronucleus formation other events that reflect cytotoxic and genotoxic damage are analyzed in exfoliated cells.

DISCUSSION

Our results show a significant increase in Micronuclei and Micronucleated cells frequency in exposed groups compared with the controls. This finding is consistent with previous studies on epithelial cells (nasal and buccal) of Formaldehyde exposed workers.[8-11] Occupational exposure to Formaldehyde evaluated in the Anatomy laboratories shows that workers are exposed to high levels of Formaldehyde. In this study, findings are very close to Holland et al. (2008 review)[12] in which buccal micronuclei/1000 cells were 0.33. A significant positive correlation between micronuclei frequencies and duration of exposure is found(r=0.7332 and p Value=0.00). This finding is similar to those of Susana Viegas (2010)[8] reported that a moderate positive correlation was found between duration of occupational exposure to Formaldehyde (years of exposure) and frequency of Micronuclei in epithelial cells (r = 0.209; p < 0.05).

Our results show that cells with abnormal nuclei that are karyolitic, karyorrhexic, pyknotic, binucleatic are persistently present in the samples from healthy individuals. Scoring of exfoliated cells with degenerative nuclei provides information about the extent of degenerative processes in a particular individual and helps accurately select normal cells in which to score mucronuclei.

CONCLUSION

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. Results obtained suggest that preventive and protective measures must be applied in order to reduce occupational exposure. Micronucleus scoring is more easy and precise. Simplicity, accuracy, multipotentiality, and large tissue applicability of the MN technology made it attractive in the past and will ensure a key role in the evaluation of mutagenicity and primary prevention in the future. An increase of micronuclei in the epithelial buccal cells of the students exposed to embalming solution vapor, may result from high exposure to the compounds contained, especially formaldehyde, and a probable human carcinogen.

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