

METANUCLEAR ABNORMALITIES IN FORMALIN-EXPOSED INDIVIDUALSPratheepa Sivasankari Natarajan¹, Sundarapandian S², Sakthivel E³¹Associate Professor, Department of Anatomy, SRM Medical College Hospital & Research Centre, SRM University, Kattankulathur, Kanchipuram.²Professor, Department of Anatomy, SRM Medical College Hospital & Research Centre, SRM University, Kattankulathur, Kanchipuram.³Assistant Professor, Department of Community Medicine, SRM Medical College Hospital & Research Centre, SRM University, Kattankulathur, Kanchipuram.**ABSTRACT****BACKGROUND**

Formalin is a genotoxic agent which causes respiratory illness on mild exposure and cancer on chronic exposure. This study provides an easy and inexpensive way to assess the nuclear abnormalities.

MATERIALS AND METHODS

Fifty individuals with different duration of exposure were included in our study. Oral squamous cells were collected by scraping the wooden spatula. The slides were fixed with methanol glutaraldehyde fixative and stained with Giemsa, May-Grunwald stains. After air drying, 1000 cells were screened for metanuclear abnormalities.

RESULTS

Metanuclear abnormalities were more in the group who had more than 15 years of formalin exposure. The mean value was 66.80. The frequency of metanuclear abnormalities were significant within the groups and between the groups also.

CONCLUSIONS

The genotoxic effect of formalin was confirmed by the increase in the nuclear abnormalities in the formalin-exposed group.

KEYWORDS

Karyolysis, Karyorrhexis, Binucleated Egg, Formalin, Genotoxicity.

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BACKGROUND

Formalin is a 40% solution of formaldehyde in water.^[1] Industrial workers, professional groups have high exposure (embalmers).^[2] Formalin causes cancer.^[3] First listed human carcinogen was Formaldehyde CAS No. 50-00-0 as per the Second Annual Report on Carcinogens (1981). Formaldehyde exposure causes head and neck cancers mainly the nasal, lymphoid and oral cancers. An excess of brain cancer mortality was found in all studies of professional group.^[4]

Among males, cancers of lung, mouth, oesophagus and stomach are the leading sites across most of the registries.^[5] Genetic damage, mutation in critical genes are related to carcinogenesis. Genetic damage evaluation, mutation and genetic instability can be used as a biomarker to assess the level of lesion,^[5] karyorrhexis (KR) and binucleated eggs (BN) (Fig. 1). Karyolysis (KL) (Fig. 2) is the nuclear change resulting from genotoxicity.^[6]

Aims & Objectives are-

To study the formalin-induced changes in the oral mucosa.
To detect the nuclear abnormalities in the formalin-exposed individuals.

MATERIALS AND METHODS

Study Design- Descriptive study.

Statistical Method- One-Way ANOVA.

SPSS Version 21 was used.

Materials- Methanol, Glacial acetic acid, Giemsa stain and May-Grunwald stain.

Inclusion Criteria

Faculties and staff working in the Department of Anatomy and the first-year students were taken as study sample that consisted of 50 subjects and divided into 5 groups as follows:

Group 1- Less than 1 year of formalin exposure.

Group 2- 1 – 5 years of formalin exposure.

Group 3- 6 - 10 years of formalin exposure.

Group 4- 10 - 15 years of formalin exposure.

Group 5- More than 15 years of formalin exposure.

Exclusion Criteria

Alcoholics, smokers, pre-malignant cases and malignant cases.

After getting the informed consent, the individuals were asked to rinse the mouth and the material was collected from the oral cavity by scraping the buccal mucosa using a clean wooden spatula. Scraped material was spread on cleaned slides and smeared. After air drying, the slides were kept in the methanol: glacial acetic acid fixative in the proportion 3:1 for 20 minutes. There fixed slides were stained with May-

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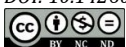
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Grunwald and Giemsa stain. They were observed for Nuclear abnormalities like Karyolysis (KL), Karyorrhexis (KR), Binucleated egg (BN) under bright field Nikon microscope under 10 x 100 magnifications. (Fig. 1, 2) Observations were recorded and tabulated. 1000 cells were screened in each person from the slides prepared and the frequency of Metanuclear abnormalities were recorded and the collected data was subjected to one-way ANOVA by using SPSS software version 21.⁽⁷⁾

RESULTS

One-way ANOVA was used to compare the frequency of nuclear abnormalities and the duration of exposure. Table 1 shows increase in the frequency of Metanuclear abnormalities in the group who had more than 15 years of exposure followed by the group who had 10-15 years of exposure. The frequency of metanuclear abnormalities was low in the group that had less than 1 year of exposure. The bar diagram shows that 66.8% of metanuclear abnormalities were more in the group that had more than 15 years of formalin exposure. (Fig 3).

DISCUSSION

Formaldehyde causes nasopharyngeal cancer and leukaemia in humans.⁽⁸⁾ The exposure level was more while doing the macroscopic examination of formalin-preserved specimens.⁽⁹⁾ Chronic exposure at young age causes delayed health effects, including cancer. In our study, the first-year students were exposed to more than 2 hours in the dissection hall for one-

year period of time. So they are more prone to health-related issues,⁽¹⁰⁾ the most important marker of cell death was nuclear abnormalities. Fragmentation of the nucleus in the cytoplasm results in Karyorrhexis (KR) (Fig. 1). Digestion of the chromatin results in a cell with no nucleus which is known as Karyolysis (KL) (Fig 2).⁽¹¹⁾

Claudia et al found that nuclear abnormalities were more in formalin-exposed individuals as stated in our study,⁽¹²⁾ relative increase in the nuclear abnormalities found in the outdoor formaldehyde exposure studies were well in correlation with our study which included the indoor formalin-exposed individuals.⁽¹³⁾ Metanuclear abnormalities were more in the workers and faculties working in the dissection hall for more than 10 years of exposure. These results were well in correlation with a study conducted by Shekawat S et al.⁽¹⁴⁾ Leon Cleres Penido et al stated that students with a more extensive workload in anatomy laboratory showed increase in karyorrhexis, the same results were seen in our study and hence we need to reduce the duration of formalin exposure in the dissection hall and should advise the students to wear mask to prevent the absorption of formalin in the nasal epithelium.⁽¹⁵⁾ Cellular injury caused by the formalin in the spray painters led to the significant increase in the nuclear abnormalities which was in correlation with our study where formalin-exposed faculties and attenders had increased number of metanuclear abnormalities.⁽¹⁶⁾

Descriptive								
Metanuclear Abnormalities	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Less Than 1	10	9.60	3.806	1.204	6.88	12.32	5	14
1 to 5	10	6.50	1.581	.500	5.37	7.63	5	10
6 to 10	10	21.20	4.492	1.420	17.99	24.41	12	27
10 to 15	10	58.70	20.651	6.530	43.93	73.47	28	83
Above 15	10	66.80	20.225	6.396	52.33	81.27	40	117
Total	50	32.56	28.487	4.029	24.46	40.66	5	117

Table 1. One-way ANOVA (Comparison Between Duration of Exposure to Metanuclear Abnormalities)

	ANOVA				
	Metanuclear Abnormalities				
	Sum of Squares	Degree of Freedom	Mean Square	F-Value	P-Value
Between Groups	31910.120	4	7977.530	45.707	0.000
Within Groups	7854.200	45	174.538		
Total	39764.320	49			



Figure 1. This Figure shows 1. Karyorrhexis and 2. Shows the Binucleated Egg

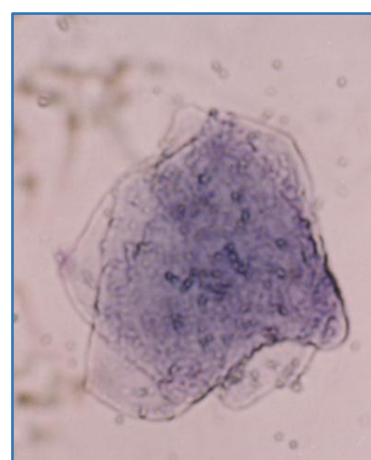


Figure 2. This Figure shows the Distorted Nuclear Membrane – Karyolysis (KL)

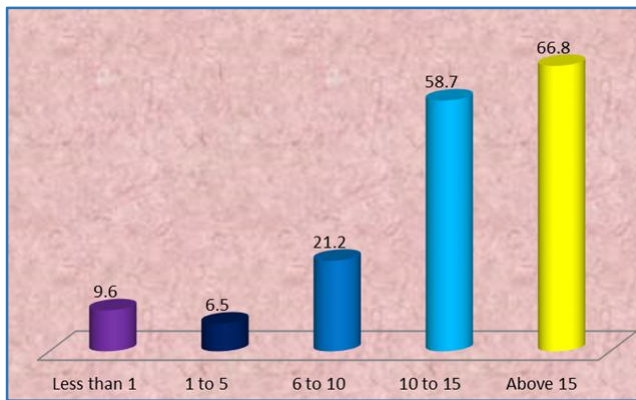


Figure 3

CONCLUSION

Nuclear abnormalities are more among the formalin-exposed individuals who had more than 10 years of exposure. Nuclear abnormalities may lead to health-related issues and cancer which is in relation with the duration of exposure. Anatomy dissection hall attenders, faculties and students are the more vulnerable groups. To prevent this, the above said groups can wear mask and the exposure time can be minimised. Apart from this metanuclear assay, the other tests like automated micronucleus assay can be done in this large population to find out the lesions at an earlier stage.

REFERENCES

- [1] National Research Council. Review of the Formaldehyde Assessment in the National Toxicology Program 12th Report on Carcinogens. Washington (DC): National Academies Press (US) 2014.
- [2] Fischer MH. The toxic effects of formaldehyde and formalin. *J Boston Soc Med Sci* 1900;5(1):18-22.
- [3] National Toxicology Program. Report on carcinogens background document for formaldehyde. http://ntp.niehs.nih.gov/ntp/roc/twelfth/2009/November/Formaldehyde_BD_Final.pdf
- [4] National Toxicology Program, Department of Health and Human Services. <https://ntp.niehs.nih.gov/go/roc14>
- [5] Indian Council of Medical Research. Ncrpblr@canceratlasindia.org. <https://ntp.niehs.nih.gov/go/roc14>
- [6] Utani KI, Kohno Y, Okamoto A, et al. Emergence of micronuclei and their effects on the fate of cells under replication stress. *Plos One* 2010;5(4):e10089.
- [7] Nersesyan A, Kundi M, Atefie K, et al. Effect of staining procedures on the results of micronucleus assays with exfoliated oral mucosa cells. *Cancer Epidemiol Biomarkers Prev* 2006;15(10):1835-40.
- [8] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. Vol.88. Lyon, France: IARC 2006.
- [9] Viegas S, Ladeira C, Gomes M, et al. Genotoxic effects of exposure to formaldehyde in two different occupational settings. In: Pesheva M, Dimitrov M, Stoycheva ST, eds. *Carcinogen*. InTech 2012. <http://www.intechopen.com/books/carcinogen/genotoxic-effects-of-occupational-exposure-to-formaldehyde>
- [10] Onyije FM, Avwioro OG. Excruciating effect of formaldehyde exposure to students in gross anatomy dissection laboratory. *Int J Occup Environ Med* 2012;3(2):92-5.
- [11] Tolbert PE, Shy CM, Allen JW. Micronuclei and other nuclear anomalies in buccal smears: a field test in snuff users. *Am J Epidemiol* 1991;134(8):840-50.
- [12] Mergener M, Rhoden CR, Amantéa SL. Nuclear abnormalities in cells from nasal epithelium: a promising assay to evaluate DNA damage related to air pollution in infants. *J Pediatr (Rio J)* 2014;90(6):632-6.
- [13] Marcon A, Fracasso ME, Marchetti P, et al. Outdoor formaldehyde and NO₂ exposures and markers of genotoxicity in children living near chipboard industries. *Environ Health Perspect* 2014;122(6):639-45.
- [14] Shekhawat S, Jadon VS, Garg R, et al. Cytogenetic effects of exposure to formalin vapours in buccal and nasal mucosae: a study in the students of anatomy and embalmers. *Adv Med Dent Res* 2015;1(1):1-5.
- [15] Pinheiro LCP, Nascimento HMS, Menegardo CS, et al. Nuclear alterations in nasal mucosa epithelial cells of students exposed to formaldehyde. *Med Segur Trab (Internet)* 2016;62(242):4-14.
- [16] Khan MR, Sellappa S. Elevated frequencies of micronuclei and other nuclear abnormalities in buccal epithelial cells of spray painters in South India. *Int J of Pharm & Life Sci* 2013;4(5):2680-4.