Journal of Cardiovascular Disease Research

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

ORIGINAL RESEARCH

Effect of age on micronucleus frequency in buccle epithelium cell through micronucleus assay in subjects exposed to formaldehyde

[1]Shabina, [2]Dr. Nand Lal, [3]Dr. Shilpa

[1]PhD Scholar, [2]Senior Professor, Department of Anatomy, SMS Medical College, Jaipur, Rajasthan, India
[3]Assistant Professor, Department of Anatomy, ESIC Medical College, Alwar, Rajasthan, India

Correspondence:
Dr. Shilpa
Assistant Professor, Department of Anatomy, ESIC Medical College, Alwar, Rajasthan, India
Email: shilpakayamkhani@gmail.com

Received: 13 October, 2022 Accepted: 19 November, 2022

Abstract

Background: Occupational exposure to formaldehyde has adverse effects on health, including acute effects (e.g., bronchial asthma), chemosensory irritation, respiratory symptoms, carcinogenic effects (e.g., nasopharyngeal cancer) and limited evidence for leukemia and sinonasal cancer and genotoxic damage**.** When a chromosome or chromosome fragment isn't absorbed into one of the daughter nuclei during cellular division, a small nucleus known as a micronucleus (MN) arises. It often serves as a sign of chromosomal mutability and genotoxic events

Aim: To study effect of age on micronucleus frequency in buccle epithelium cell through micronucleus assay in subjects exposed to formaldehyde.

Methods and Materials: In this study 100 study participants were included. They were divided into two groups. Case group and control group. Each group consisted of 50 participants. Case group included study participants exposed to formaldehyde and while control group consisted of non exposed participants. In both the case group and the control group, the study participants' ages were noted Buccal cells were scraped off the inside of the cheek after a thorough mouthwash. The cells underwent two centrifugal separations after being placed into centrifuge tubes with 5ml of 0.9% saline. The supernatant waste and fixative (3:1 Methanol and acetic acid) were both added. Using a Pasteur pipette, the cells were put onto a chilled slide before being air dried. Two slides were produced for each sample. After being labeled, air dried and fixative-fixed for 20 minutes, the slide was stored for 24 hours before staining. To mark the slides, Giemsa staining solution was used.

Results: Mean age of case group was 27.86 ±10.11 years where as in control group it was 31.12 ±10.16 years. When unpaired t test used there was no significant difference was found between case and control group w.r.t. of age. (p=0.111).Maximum participants in case and control groups (70% & 56% respectively) were in age group ≤30years, while minimum participants (2% and 4% respectively) were in age group 51-60 years. When there was analysis of effect of age on MN count then significant positive correlation of age was present with MN count/500 cells in both case and control group. (p=0.003 & p=0.0060 respectively).

Conclusion: In this study significant positive correlation of age was present with MN count/500 cells in both control and case group.

Keywords: Age, micronuclei, microassay, formaldehyde**.**

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

Introduction

Formalin, a solution used in manufacturing formaldehyde, is produced for commercial use. Formalin typically comprises 7% dissolved formaldehyde by weight and 10% methanol, which prevents excessive oxidation and polymerization of the solution. Formol, another often produced solution, can be a formaldehyde-in-water solution at a concentration of 10%. In embalming fluid and medical labs, formalin is often utilised as a tissue preservative or bactericide. In their daily jobs, nurses, medical workers, and lab scientists are frequently exposed to formaldehyde.[1,2]

It is a frequently used preservative in mortuaries and medical labs. Workplace exposure to formaldehyde can have a variety of negative effects, 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. Formaldehyde is an allergy and irritant that can cause respiratory tract irritation, occupational dermatitis, conjunctivitis, and mucosal irritation.[3,4,17,18]

When a chromosome or chromosome fragment isn't absorbed 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.[5,6,21,22]

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. To check for the presence of these structures and gauge their frequency in cells exposed to certain substances or under stressful circumstances, numerous micronucleus assays have been created. The MN assay frequently exhibits both clastogenic and aneugenic effects because MN is derived from chromosomal fragments or complete chromosomes that lag behind during anaphase.[7,8,19,20]

The superficial buccal cells of the stratified squamous cell epithelium are continuously lost and are replaced by cell division. Chromosome fragments or whole chromosomes may be lost during mitotic anaphase and appear as minute nuclear particles, or micronuclei, in the cytoplasm of daughter cells when these cells divide.[9,10,23,24] Only after the cells have grown and migrated to the surface can the cytogenetic changes from the exfoliated cells be assessed. The micronuclei assay (MA) on exfoliated buccal cells is a novel genotoxicity technique that exhibits potential for the analysis of epithelial carcinogens. Micronuclei are excellent internal dosimeters for measuring tissue-specific genotoxic damage in individuals exposed to carcinogenic combinations.This study investigated the effect of cumulative formaldehyde exposure on the frequency of micronuclei in buccal epithelial cells.[11,12]This study was carried out to analyse effect of age on micronucleus frequency in buccle epithelium cell through micronucleus assay in subjects exposed to formaldehyde.

Method and Materials

In this study 100 study participants were included. They were divided into two groups. Case group and control group. Each group consisted of 50 participants. Case group included study participants exposed to formaldehyde and while control group consisted of non exposed participants. In both the case group and the control group, the study participants' ages were noted.

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

Criteria for inclusion

Study participants who are willing to participate and are at least 18 years old.
People who served as controls and were never exposed to formaldehyde.
Test subjects exposed to formaldehyde for a minimum of one year and a maximum of thirty years

Criteria for exclusion

People who refuse to participate in the study.
People who work in the paint or pesticide industries, which are recognised as carcinogens, are excluded from both the research and control groups.
People who routinely undergo X-rays and other radiation treatments.
Patients who have previously received cancer treatment.

Procedure of collection of sample and criteria for analysis

Buccal cells were scraped off the inside of the cheek after a thorough mouthwash. The cells underwent two centrifugal separations after being placed into centrifuge tubes with 5ml of 0.9% saline. The supernatant waste and fixative (3:1 Methanol and acetic acid) were both added. Using a Pasteur pipette, the cells were put onto a chilled slide before being air dried. Two slides were produced for each sample. After being labelled, air dried, and fixative-fixed for 20 minutes, the slide was stored for 24 hours before staining. To mark the slides, Giemsa staining solution was used. The following criteria for scoring were applied to the Tolbert et al.[9] criteria that were used to identify the MN: The MN should be rounded and smooth. The accompanying nucleus's diameter should be roughly one-third that of the MN. In terms of lighting, the MN should be both bright and dark. The staining intensities of the MN and nucleus should be comparable. A nucleus should be able to be made out of the MN texture. The focal plane must be identical to that of the nucleus. There is no overlap or bridge with the nucleus.

Equipments used

Xylene, centrifuge tubes, saline, fixative (3:1 methanol and acetic acid), Pasteur pipette, 100% alcohol, Giemsa solution
Regression equation obtained was as follow-
Mn count/500 cells= - 1.2749+0.1101(age) + 6.3688(exposure to formaldehyde)

Statistical Analysis

Multivariate regression analysis of many independent factors for MN count/500 cells was carried out using a step-by-step methodology. The probability of leaving the model was kept at >0.10, whereas the likelihood of staying in the modal was kept at 0.05. The model included information on age, sex, smoking and drinking habits, nutrition and family history of diabetes, hypertension, and formaldehyde exposure. There were only two independent variables left in the model: age and formaldehyde exposure. The coefficient of determination for the model, or R2, was 0.6471. The analysis of variance revealed the significant prediction of the model. ($p<0.001$)

Results

Mean age of case group was 27.86 ±10.11 years where as in control group it was 31.12 ±10.16 years. When unpaired t test used there was no significant difference was found between case and control group w.r.t. of age. ($p=0.111$).(Table 1)Maximum participants in case and control groups (70% & 56% respectively) were in age group ≤30years, while minimum participants (2% and 4% respectively) were in age group 51-60 years. On

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

application of chi- square test both the groups were found comparable w.r.t. age distribution. (p= 0.464).(Table 2)

Mean MN /500 cells of case group was 8.16 ± 3.46 years where as in control group it was 2.15± 1.37 years. When unpaired T test used there was significant difference was found between case and control group w.r.t. of Mean MN/500 cells. (p< 0.001).Mean MN /500 cells of case group was 8.16 ± 3.46 years where as in control group it was 2.15± 1.37 years. When unpaired T test used there was significant difference was found between case and control group w.r.t. of Mean MN/500 cells. (p< 0.001). (Table 3)

When there was analysis of effect of age on MN count then significant positive correlation of age was present with MN count/500 cells in both case and control group. (p=0.003 & p=0.0060 respectively).(table 4)

Table 1- Comparison of age of participant between case and control.

Age (years)							
Group	**N**	**Mean**	**SD**	**Median**	**Min.**	**Max.**	**'p' Value***
Case	50	27.86	10.11	20	20	57	
Control	50	31.12	10.16	28.5	18	60	
Control	50	75.80	3.82	76	64	86	0.111

Table 2: Distribution of study participants according to different age groups

Age Group (Years)	Group				Total	
	Case		Control			
	No.	**%**	**No.**	**%**	**No.**	**%**
≤30	35	70.00	28	56.00	63	63.00
31-40	8	16.00	15	30.00	23	23.00
41-50	6	12.00	5	10.00	11	11.00
51-60	1	2.00	2	4.00	3	3.00
Total	50	100.00	50	100.00	100	100.00

Table 3- Comparison of Mean MN/500 cells of participant between case and control.

	Group	**N**	**Mean**	**SD**	**Median**	**Min.**	**Max.**	**'p' Value***
Mean MN/500	**Case**	50	8.16	3.46	7.75	3	18.5	
	Control	50	2.15	1.37	2	0	6	<0.001

Table 4– Correlation of age with MN count/500 cell in different groups.

		N	**Correlation Coefficient r**	**'p' Value**	**95% CI for r**
Age	**Case**	50	0.493	0.0003	0.2483to0.6780
	Control	50	0.384	0.0060	0.1177to0.5980
	Total	100	0.153	0.1292	-0.04502to0.3390

Discussion

Health Hazard Evaluation (HHE) report reveals that repeated eye irritation, drowsiness, nasal congestion, fatigue and headache are noted in people who are exposed to formaldehyde levels of 0.2ppm. There is variation among individuals regarding sensitivity towards formaldehyde and specifically in terms of an individual's tolerance and susceptibility to acute exposures of the compound.[11,21] Formaldehyde and Facts about Health Effects prepared by formaldehyde Epidemiology, Toxicology and Environmental Group states that the lowest level at which many people can begin to smell formaldehyde is about 0.3 ppm. An experts panel review of over 150 published studies found that eye irritation doesn't become significant until around 1 ppm and moderate to severe eye, nose and throught irritation occurs at 2 to 3 ppm.[12,22]

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

Speit G et al studied the local genotoxic effects of formaldehyde in humans by the micronucleus test with exfoliated buccal mucosa cells. Buccal smears were prepared one week before the beginning of the study (control 1), at the beginning of the study before the primary exposure (control 2), at the top of the exposure period of 10 days and seven, 14, and 21 days thereafter. Two thousand cells per information were analyzed for the presence of micronuclei (MN) and therefore the frequency of MN per 1000 cells made up our minds on slides coded by an independent quality-assurance unit. No significant increase within the frequency of MN was measured at any time conversion the top of the exposure. This study, which was performed under GLP-like conditions, clearly indicates that FA doesn't induce MN in buccal mucosa cells after peak exposures up to 1 ppm and cumulative exposure of 13.5 ppm over period.[13,25]

Solange Costa et al studied Micronucleus frequencies in buccal cells in formaldehyde exposed workers. Formaldehyde (FA) could be a high-volume production chemical produced worldwide with an oversized range of commercial and medical uses. Listed, since 2004, by IARC as a personality's carcinogen, FA status was recently revised by the U.S. government who reclassified this compound as known to be a person's carcinogen. Both reclassifications are supported sufficient evidence of carcinogenicity from epidemiologic studies, supporting data on mechanisms of carcinogenesis and experimental evidence in animals. the very best level of human exposure to FA occurs in occupational settings.[10,14]

Very few studies have been conducted to analyse the effect of age on affected the frequency of micronuclei in buccal epithelial cells after exposure to formaldehyde.

In this investigation, we looked at how age affected the frequency of micronuclei in buccal epithelial cells after exposure to formaldehyde. It was found that both the case and control groups in this investigation showed a strong positive connection between age and MN count/500 cells. Costa et al. also found In exposed groups ($r=0.257$, $P< or = 0.05$) and buccal cells ($r=0.257$, $P< or = 0.05$), there was a strong positive connection between age and MN frequency.[10] In order to evaluate the impact of various factors affecting MN frequency, Bonassi S et al. reported a study on 5424 participants with buccal MN values acquired from 30 laboratories around the world. The age trend was highly significant ($p<0.001$) and consistent with the current study.[11,15,16] Higher micronuclei frequencies have been seen in older children, according to a study by Vleminckx C, indicating a relationship between exposure and age. There was an increase in micronuclei by 240% in children living near a chemical disposal site.[12]

In the present study, mean and standard deviation of age of case group was 27.86±10.11 years where as in control group it was 31.12±10.16 years. 50 participants in case 70% (35) were below 30 years age group, 16% (8) were in 31-40 age group, 12% (6) were in 41-50 age group, 2% (1) were in 51-60 age group. P value was greater than 0.05 so age is not a determining factor between case and control for formaldehyde exposure in the present study. ($p=0.111$).

Susana pastor[13] included 50 people in exposed group and 66 people in control group in their study of cytogenetic analysis in buccal mucosa of Greek farmers reported that the mean age of exposed group (50) was 42.98±1.60 and in control group (66) 43.94±1.11. Though significant relation could not find in their study. Costa et al conducted a study on 80 women of Portugal including 38 females working in anatomy and pathology laboratory and 42 in administrative office without occupational exposure to formaldehyde. Age of exposed group was found to be 38.90 ± 11.99 and 39.68 ± 8.49 for non-exposed group. no significant correlation was found in the study between age and formaldehyde exposure. ($P=0.074$).[10]

Susana Viegas et al conducted a study on 80 workers exposed to formaldehyde during their working hours and 85 non-exposed subjects from Portuguese population.[14] They suggested that age of exposed subject ranged between 20-55yrs with an average of 33.87±8.262 and

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

35.74±9.470 for unexposed subject age ranging from 19-56 years. No significant relation was found in their study (p=0.180) This suggest that formaldehyde exposure is not related to age, since person cannot escape daily exposure of formaldehyde in form of automobile smoke, furniture and other various things in their day to day life.

Present study showed significant difference between micronucleus per 500 cells in cases and control group with P<0001. Average MN frequency /500 cell was 8.16 ± 3.46 years where as in control group it was 2.15± 1.37 years. Similarly, Viegas et al reported higher frequency of MN in occupationally exposed subject than control group (P<0001). Average MN frequency in Control reported 0.13 ± 0.48 and 0.88 ± 1.69 in cases.[14] Shekhawat S et al reported in a study that average frequency of Micronuclei in buccal cells with pap staining was 0.365±0.154 in cases and 0.0734 ±0.018 in control. this difference was statistically significant.[15] Costa et al found significant association between non exposed and exposed cases for micronucleus frequency during assessment of genotoxic effects of formaldehyde in portugese population.[10]

A review on association between the occupational exposure to particles, formaldehyde and heat and nasopharyngeal carcinoma (NPC) by Armstrong R W et al studied that NPC wae associated with the exposure to chemicals, industrial heat and cigarette smoke but there was no association between of NPC and formaldehyde.[23]Selim Mohamed Elshaer and Madiha Awad Elsayed Mahmoud studied Toxic effects of formalin-treated cadaver on medical students, staff members, and workers within the Alexandria Faculty of Medicine. This study highlighted the irritating action of formalin treated cadavers on medical students, which necessitate re-evaluation of the concentration of formalin, proper ventilation within the dissecting rooms, and assessment of working practices conditions at the department of Anatomy at the Alexandria Faculty of Medicine. Moreover, it's highly recommended to conduct comparative cross-sectional studies of enormous sample size to be able to generalize the conclusion that chronic exposure to formalin at the FOM is significantly related to systemic disorders and disruption of the hematopoietic system. Pathologists within the AFM, and workers in morgue at university hospitals may be included in the formalin exposed group.[24]

Conclusion

In this study significant positive correlation of age was present with MN count/500 cells in both control and case group.

References

1. Hildesheim A, Dosemeci M, Chan CC, Chen CJ, Cheng YJ, Hsu MM, Chen IH, Mittl BF, Sun B, Levine PH, Chen JY, Brinton LA, Yang CS: Occupational exposure to wood, formaldehyde, and solvents and risk of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev 2001; 10:1145-1153
2. Coggon D, Harris EC, Poole J, Palmer KT: Extended follow-up of a cohort of British chemical workers exposed to formaldehyde. J Natl Cancer Inst 2003; 95:1608-1615
3. Hauptmann M, Lubin JH, Stewart PA, Hayes RB, Blair A: Mortality from solid cancers among workers in formaldehyde industries. Am J Epidemiol 2004; 159:1117-1130.]
4. International Agency for Research on Cancer: Formaldehyde, 2- Butoxyethanol and 1-tert-Butoxypropan-2-ol. Lyon: IARC 2006].
5. Fenech M, Kirsch M, Natarajan A T et al. Molecular mechanisms of Micronucleus, Nucleoplasmic bridge and nuclear bud formation in mammalian and human cells. Mutagenesis 2011; 226(1):125-132.

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

6. TianY , Ishikawa H , Piao FY , Yamamoto H , et al. Micronucleus assay of human lymphocytes: A comparison of cytokinesis and human capillary blood lymphocyte method. J Occup Health 2003; 45:408-409.
7. Norppa H , Falck GCM , What do human micronuclei contain ? Mutagenesis 2003 May; 18(3):221-33.
8. Wunnapuk K , Ruangyuttikarn W , Anusri Y and Prapamontol . Increase In Epithelial Buccal Cell Micronuclei In students Exposed To Embalming Solution Vapour . Chiang Mai Med J 2008;47(3):115-123.
9. Tolbert PE, Shy CM Allen JW. Micronuclei and other nuclear anomalies in buccal smears: A field test in snuff users. Am J Epidemiol.1991;134:840-50.
10. S. Costa, F. Brandão, M. Coelho, C. Costa, P. Coelho, S. Silva, B. Porto & J. P. Teixeira. Micronucleus frequencies in lymphocytes and buccal cells in formaldehyde exposed workers. WIT Transactions on Biomedicine and Health 2013; 16:83-94.
11. 11.Bonassi S, Coskun E, Ceppi M, Lando C, Bolognesi C, Burgaz S.The HUman MicroNucleus project on eXfoLiated buccal cells (HUMN(XL)): the role of lifestyle, host factors, occupational exposures, health status, and assay protocol. Mutat Res. 2011 Nov-Dec; 728(3):88-97.
12. 12.Vleminckx C., Klemans W., Schriewer L., Joris I., Lijsen N., Ottogali M., Pays A., Planard C., Rigaux G., Ros Y., et al. Performance of cytogenetic biomarkers on children exposed to environmental pollutants. Toxicol. Ind. Health. 1997; 13:219–230
13. 13.Pastor S, Gutierrez S. Creus A, Xamena N, Piperakis S and Marcos R. Cytogenetic analysis of Greek farmers using the micronucleus assay in peripheral lymphocytes and buccal cells. Mutagenesis 2001;16(6):539-545.
14. Viegas S, Ladeira C, Nunes C, Malta-Vacas J, Gomes M, Brito M, Mendonca P, Prista J. Genotoxic effects in occupational exposure to formaldehyde: A study in anatomy and pathology laboratories and formaldehyde-resins production. J Occup Med Toxicol. 2010 Aug 20;5(1):25. doi: 10.1186/1745-6673-5-25. PMID: 20727169; PMCID: PMC2933706
15. Shekhawat S, Jadon VS, Garg R and Mogra K. 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.
16. Jia-Kun Chen 1, Charlene Wu 2 and Ta-Chen Su 1,3,4,* Positive Association between Indoor Gaseous Air Pollution and Obesity: An Observational Study in 60 Households Int. J. Environ. Res. Public Health 2021; 18: 114-47.
17. Jeanne Pawitan et al. The micronucleus test: A method used in mutagenicity testing. Medical Journal of Indonesia 1995;4(2):65-71.
18. Titenko-Holland N, Levin AJ, Smith MT, Quintana PJE, Boeniger M, Hayes R et al . Quantification of epithelial cell micronuclei by fluorescence in situ hybridization (FISH) in mortuary science students exposed to formaldehyde. Mutant Res 1996;371(3-4):237-48.
19. Nersesyan AK, Andllin AI. The micronucleus assay in exfoliated human cells: A mini – review of papers from the CIS. Tsitol Genet 2007 March-Apirl;41(2):56- 66.
20. Suruda A, Schulte P, Boeniger M. Cytogenetic Effects of Formaldehyde Exposure in Students of Mortuary Science. Cancer Epidemiol Biomarkers prev 1993; 2:453- 460
21. Kaiser EA and Sylvain DC. Health Hazard Report. [online] 1994 [cited 1994 Nov] Available from URL:http://www.cdc.gov/niosh/hhe/reports/pdfs/1994-0023- 2473.pdf (Access date 13/03/22).
22. Formaldehyde and Facts About Health Effects [online] 2002 [cited 2002 Aug]. Available from URL:http://www.2.dupont.com/plastics/en_ US/assets/ downloads/ processing/ FETEG_Facts.pdf (access date 03/03/22).

ISSN: 0975-3583,0976-2833 VOL13, ISSUE 08, 2022

23. Armstrong RW, Imrey PB, Lye MS, Armstrong MJ, Yu MC, Sani S: Nasopharyngeal carcinoma in Malaysian Chinese: occupational exposures to particles, formaldehyde and heat. Int J Epidemiol 2000; 29:991-998.
24. Selim Mohamed Elshaer and Madiha Awad Elsayed Mahmoud. Toxic effects of formalin-treated cadaver on medical students, staff members, and workers in the Alexandria Faculty of Medicine. Alexandria Journal of Medicine 2017;53: 337– 343.
25. Speit G, Schmid O, Frohler -Keller M, Lang I and Triebig G. Assessment of local genotoxic effects of formaldehyde in humans measured by the micronucleus test with exfoliated buccal mucosa cells. Mutat Res 2007 Mar 5;627(2):129-35