Formaldehyde exposure in medical students: a short period of contact causes DNA damage and instability

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Abstract— *Occupational exposure to formaldehyde (FA) has been related to adverse outcomes. However, a short period of exposure has never been assessed in terms of evaluating DNA. This study conducted on 39 medical students exposed to FA in a university laboratory of human anatomy and aimed to analyze the relationship between FA exposure and DNA damage. The buccal micronucleus cytome assay (BMCyt) was used to evaluate the students at four time points: before FA exposure, after four months of FA exposure, after eight months of FA exposure and after three months without FA exposure (remission period). Pyknotic cells, karyolitic cells, karyorrhetic cells, condensed chromatin, binucleated cells, basal cells differentiated cells, micronucleated cells and nuclear bridges were enumerated. This study shows that FA exposure caused genomic instability in all periods and the remission period was not sufficient to reverse all damage. Thus, prolonged occupational exposure to FA not only causes DNA damage but a shorter exposure period can have the same effect.*

Keywords— Buccal MNi, DNA Damage, Formaldehyde Exposure, Genome Instability, Medical Students

I. INTRODUCTION

Formaldehyde (FA) is a ubiquitous chemical used in the furniture industry, laboratories and hospitals. As a result, a large number of people are exposed to FA in the environment and/or workplace. It has been classified by IARC as a potent human carcinogen[1] due to the increased incidence of nasopharyngeal cancer in workers exposed to FA.[2] The major route of FA absorption is through inhalation (>90%), mainly affecting the upper airways.[3]

Buccal micronucleus cytome (BMCyt) assay is widely used in occupational, environmental, lifestyle and nutritional studies.[4,5] This assay is useful for assessing cancer risk following exposure to a genotoxic carcinogen[6] such as formaldehyde. The BMCyt assay is a cost-effective, minimally invasive test for evaluating genomic damage, cell death and cytostasis.[7,8]

Cadavers in gross anatomy laboratories emit formaldehyde vapors during dissection, and significant exposure of staff and students has been reported.[9] In this way, anatomists, pathologists, embalmers and students are exposed to this toxin for prolonged periods of time. Many studies have shown a relationship between formaldehyde exposure and toxicity.[9,10] However, no studies have assessed the relationship between FA and DNA damage in the buccal cells of medical students, as this type of FA exposure is considered to be short-term.

Given the current lack of studies associating exposure to FA and DNA damage with brief periods of FA exposure, this study aimed to evaluate medical students exposed to FA in a university laboratory of human anatomy and to find out relationship between FA exposure and DNA damage.

II. METHODOLOGY

This follow-up type of comparative study was conducted on medical students in Department of Genetics, Universidade Estadual do Oeste do Parana, Brazil.

2.1 Study population

All subjects provided signed informed consent following study approval by the Ethics Committee (CAAE:10734912.4.0000.0107). The exposed group consisted of 39 medical students exposed to FA on a weekly basis, with more than 300 hours of exposure over the course of one year. Four sample collections were performed throughout this year: prior to FA exposure (collection 1), after four months of FA exposure (collection 2), after eight months of FA exposure (collection 3), and after three months without FA exposure (collection 4, remission period).

2.2 Questionnaire

At the time of sample collection, the subjects filled out a questionnaire to provide data on personal identity, family history, smoking habits, alcohol consumption, as well as the subject's medical and genetic history. Only individuals without a family history of cancer and without any chronic disease were included. Smokers were defined as those who had smoked at least 100 cigarettes during their lifetime, or who were smoking occasionally or every day at the time of recruitment; ex-smokers were subjects who had stopped smoking for at least one year prior to sample collection.[11] To evaluate alcohol consumption, the study subjects were classified into three categories: non-consumer, defined as no alcohol consumption or social alcohol consumption; moderate drinking, defined as consuming up to 1 cup (~100 ml) of alcohol per day, or more than one alcoholic beverage on weekends; high consumption, defined as the consumption of more than 1 liter of light alcoholic beverage (beer, wine, or cider), or two alcoholic beverages containing spirits (rum, vodka, or whiskey) per day, for at least six years.[12] High consumers of alcohol were excluded from the study.

2.3 Buccal micronucleus cytome assay (BMCyt)

The collection and storage of oral mucosa materials were performed according the protocol described by Thomas et al.[8] Briefly, cells of the buccal mucosa were collected by scraping the inner cheek with a sterile swab that was placed in a Falcon tube containing 3 mL of saline solution. The samples were centrifuged at 1000 rpm for 5 minutes and fixed using a glacial acetic acid and methanol solution (1:3, v/v), before centrifugation at 1000 rpm for an additional 5 minutes. The same fixative was added to the Falcon tube, which was stored in the refrigerator for 24 hours. For staining, suspended cells were dropped onto clean, cold slides, which were allowed to dry at room temperature for more 24 hours. Then, the slides were treated with 5N HCl for 10 minutes. Distilled water was used to wash the slides. After drying, the slides were staining with Schiff's reagent for 90 minutes and counterstained with 0.5% FastGreen for 3 minutes. One thousand cells per slide from each individual at each collection time point were analyzed using an optical microscope to count the number of pyknotic cells (PYC), karyolitic cells (KYL), karyorrhetic cells (KHC), cells with condensed chromatin (CC), binucleated cells (BN), basal cells and differentiated cells (DIFF). More than 2,000 differentials cells per slide were analyzed to

enumerate micronucleated cells (MNi), nuclear bridges and nuclear buds (called nbud). In total, 2,000 cells were analyzed in the first analysis and 4,000 cells were analyzed in the second analysis, per individual (Figure 1).

2.4 Statistical analysis

Data were statistically analyzed by one-way ANOVA for repeated measures (ANOVA RM); when a significant difference was found between groups, the Tukey test was applied to compare different time points with each other. Collection 1 was considered to reflect the normal condition and $p<0.05$ was set cut off for significance. To analyze the confounding variables gender and smoking, one-way ANOVA was used considering a significance level of 5%.

III. RESULTS

Study population in all four group were without statistically significant differences regarding age, sex and tobacco use (smokers vs. nonsmokers) in the analysis.

Table 1
Demographic characteristics of the studied population

	N	Gender		Age (years)	Smokers		No-smokers		Alcohol consumption			
		M	F		M	F	M	F	NC		MC	
									M	F	M	F
Collection 1	39	21	18	20±3.23	7	2	14	16	7	3	14	15
Collection 2	39	21	18	20±3.15	7	4	14	14	3	3	18	15
Collection 3	39	21	18	21±3.28	9	4	12	14	2	0	19	18
Collection 4	39	21	18	21±3.21	9	4	12	14	1	0	20	18

M: male; F: female; NC: non-consumer; MC: moderate consumer.

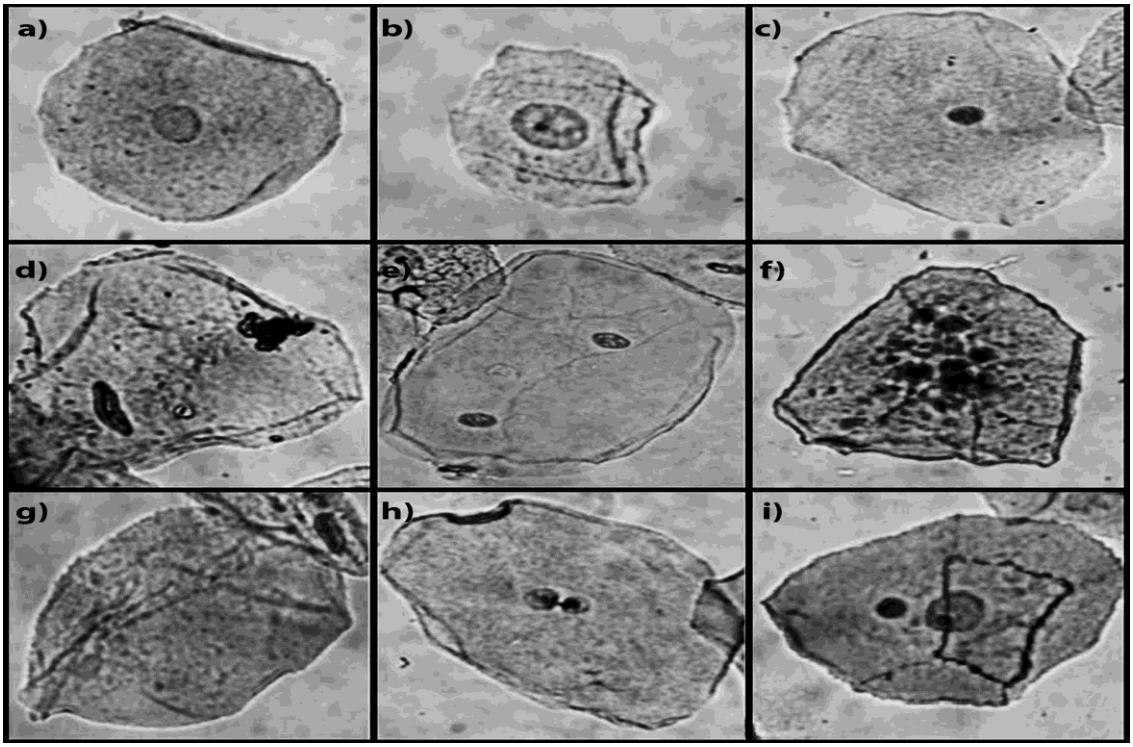
For comparison in this study, collection 1 (prior to FA exposure) was considered to reflect the normal condition. BN, KHC, nuclear bridges, CC, and MNi increased as per to duration of exposure ($p<0.05$). KYL changes were of short duration, increasing only at collection 2 ($p<0.05$), followed by a return to normal values ($p>0.05$). PYC showed a long-term effect, with an increase at collection 3 ($p<0.05$). Only KHC, nbud, and MNi showed recovery after the cessation of FA exposure, as the data from collection 4 (after three months of no FA exposure) were not different from those at collection 1 ($p>0.05$). A three-month period without exposure was not sufficient for BN, PYC and CC to return to baseline levels, as the values at collection 4 were statistically higher than those at collection 1. DIFF decreased ($p<0.05$) according to the duration of exposure, probably caused by alterations in the other parameters. Basal cells were reduced ($p<0.05$) in collection 2, normal in collection 3, and increased in collection 4 (Table 2). This could be explained by increases and decreases in the other parameters

Table 2
Mean and standard error of the results of the four collections per 1000 cells.

Cell types	Collection 1	Collection 2	Collection 3	Collection 4
Basal	0.0219±0.0013a	0.0143±0.0009b	0.0224±0.0012a	0.0386±0.0028c
Differentiated	0.0015±0.0002a	0.0031±0.0002b	0.0045±0.0003c	0.0028±0.0002b
Binucleated	0.0096±0.0009a	0.0260±0.0014b	0.0203±0.0013c	0.0223±0.0020b,c
Condensed chromatin	0.0017±0.0002a	0.0067±0.0006b	0.0021±0.0003a	0.0039±0.0005a,c
Karyolytic	0.0004±0.0001a	0.0008±0.0001b	0.0017±0.0002c	0.0005±0.0008a
Karyorrhetic	0.4640±0.0012a	0.4490±0.0018b	0.4470±0.0019b	0.4250±0.0034c
Micronucleated	0.0001±0.0000a	0.0005±0.0000b	0.0015±0.0001c	0.0002±0.0001a
Pyknotic	0.0008±0.0001a	0.0005±0.0001a	0.0018±0.0002b	0.0074±0.0008c
Nuclear bridge	0.0002±0.0002a	0.0007±0.0000b	0.0010±0.0001c	0.0001±0.0001a

**** Different letters in the same row correspond to a statistically significant difference***

Figure 1: Cell types observed in analyses



a) Differentiated cell; b) Basal cell; c) Pyknotic; d) Condensed chromatin; e) Binucleated cell; f) Karyorrhetic; g) Karyolitic; h) Nuclear bridge; i) Micronucleated cell.

IV. DISCUSSION

Peteffi et al.[13] showed an increase in DNA damage in workers following exposure to low levels of FA in a furniture manufacturing facility. Saowakon et al.[14] showed a higher concentration of FA in the air during the dissection of cadavers, and decreases in pulmonary function in technicians and students. These are some of the many studies the have assessed FA exposure in humans and its consequences. These findings are disturbing, since the 12th report on carcinogens produced by the National Toxicology Program showed convincing epidemiological evidence of an association between FA exposure and nasopharyngeal and sinonasal cancer in humans.[15]

It is known that the presence of micronuclei and other nuclear abnormalities in oral cavity cells may be associated with genetic defects from exposure to genotoxic agents.[7] In our study, it was observed that

increased exposure time worsened genetic damage. Thus, we suggest that damage induced by FA may accumulate with prolonged exposure, and that chronic exposure increases the frequency of MNi, nbud, and BN. These cell changes are positively correlated with DNA damage. The buccal mucosa is one of the most damaged by the inhalation of FA.[16] This increase in DNA damage indicates genomic instability and cytokinesis defects, even after a short period of exposure, as observed in the samples from collection 2. A period without exposure to FA is important to reverse some of this damage, although complete reversal was not observed in this study, as MNi and nbud were statistically higher in samples from collection 4. Moreover, the BN frequency remained high, indicating bioaccumulation and a long-lasting effect of FA. Costa et al.[17] evaluated workers exposed to formaldehyde in pathology laboratories and observed a high frequency of MNi in lymphocyte cultures and genotoxic damage demonstrated by the comet assay. Similarly, Hauptmann et al.[18] evaluated the medical records of deceased funeral industry professionals and found a significant increase in the number of deaths from myeloid leukemia according to the duration of exposure to formaldehyde. Ladeira et al.[19] showed a positive correlation between FA exposure and increases in genotoxic biomarkers, such as MNi. Taken together, these results demonstrate the consequences of FA contact, mainly over a long period of exposure.

This present study also showed an increase in CC and karyorrhetic cells associated with exposure to formaldehyde. Over a short period of time (four months of exposure), KYL was found to be increased; over a longer period of time (eight months of exposure), PYC was found to be increased. Thomas et al.[8] observed that these changes are indicative of cell death. The induction of apoptosis could be related to significant DNA damage[20] and may occur by specific endogenous and exogenous stimuli under normal physiological conditions or following exposure to genotoxic agents. Derka et al.[21] observed, in animal models, that cellular proliferation and apoptosis rates are high in the early stages of tumorigenesis. It is rather disturbing that even a short period of exposure to formaldehyde causes DNA damage and cytotoxic deregulation. Ke et al.[22] showed that high concentrations of FA inhibit cell growth. This interference in the cell cycle plus DNA damage could be responsible for the high number of cells in various stages of death. Following FA exposure, homeostasis is compromised, so cells are forced to die in an attempt to revert to normal conditions. It is especially worrying that the frequencies of CC and PYC were not significantly reduced after the cessation of FA exposure (collection 4).

This study showed a significant increase in the frequency of DIFF, proportional to the increase in nuclear aberrations. Regarding the frequency of basal cells, first a decrease was observed, followed by an increase in samples from collection 3. This suggests a high rate of mitosis as the oral tissue attempts to regenerate.

In the literature, is common to find information on FA exposure, but few studies have evaluated DNA damage following different periods of exposure. It is known that every human responds differently to genotoxic agents. In our study, we evaluated the same subjects over time, which confirms that FA exposure contributes substantially to DNA damage since genetic variability was not a confounding factor.

Taken together, these data show that FA exposure, even over a short period of time, has negative effects on genome instability, increases DNA damage, and alters cytokinesis. Moreover, a remission period (three months without FA exposure) was found to be insufficient to normalize this damage. The use of FA in laboratories should be revaluated, as the literature is full of reports associating FA exposure and

the development of cancer in occupational workers. It has now been found that a short period of contact can generate as much damage as a long period of exposure.

V. CONCLUSION

A short period of exposure to FA was found to induce genome instability and DNA damage. A remission period of three months was not sufficient to normalize this damage. Thus, use of FA in laboratories should be revaluated and more studies should be performed on short-term exposure, since the damage is similar to that found after a long period of FA exposure.

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CONFLICT

None declared till date.

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