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Short Communication

Cytogenetic damage in the buccal epithelium of Brazilian aviators occupationally exposed to agrochemicals

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ABSTRACT. The frequency of micronuclei in both buccal cells and peripheral blood lymphocytes is extensively used as a biomarker of chromosomal damage and genome stability in human populations. We examined whether prolonged exposure to complex mixtures of

pesticides leads to an increase in cytogenetic damage. The exposed group comprised 50 agricultural aviators, mainly from Central and Southeast regions of Brazil, who had inhaled agrochemicals for more than 10 years without personal protection equipment; the control group consisted of 17 men from the same regions, without indication of exposure to pesticides, There were three times higher frequencies of micronuclei ($P < 0.05$) and 2.5 times higher frequencies of binucleated cells in the aviators when compared to controls. However, cytotoxic alterations such as broken eggs and karyorrhexis did not present statistically significant differences between the exposed and control groups. Therefore, diverse agrochemicals used to combat pests in agriculture possess genotoxic effects in the oral mucosa of the agricultural pilots, as showed in this study.

Key words: Genotoxic agents; Mutagenesis; Biomonitoring; Occupational exposure

INTRODUCTION

Occupational exposure to mutagenic agents such as pesticides and fertilizers has increased in developing countries. However, there is no effective control and biomonitoring in the use of such products. Of special concern are their undesirable health effects in humans, other than acute intoxications. Although pesticides are important for the control of various pests, their utilization could be harmful mainly to occupationally exposed human beings (Delgado and Paumgartten, 2004).

According to Lucero et al. (2000), human cytogenetic biomonitoring studies carried out using somatic cells are considered useful tools to evaluate the possible genotoxic effects of a defined exposure. In this context, the biomonitoring of populations exposed to mutagenic and carcinogenic agents could be carried out by the micronucleus (MN) assay (da Cruz et al., 1994; Bonassi et al., 2003; Chen et al., 2006). In the present study, we used the MN assay because of its advantages for the screening of cytogenetic damage due to potential environmental mutagens (Lucero et al., 2000). Micronuclei are acentric chromosome fragments or whole chromosomes left behind during mitotic cell division and appear in the cytoplasm of interphase cells as small additional nuclei. The MN test is faster and easier than metaphase analyses and it can be used both in vivo and in vitro in a variety of cells (da Cruz et al., 1994; Lucero et al., 2000; Chen et al., 2006; Ünyayar et al., 2006).

There are some criteria that must be observed for the correct classification of micronuclei, such as: a size less than one-third the diameter and at least one-sixth of the main nucleus; the MN must not be refractive; its staining must show the same color as the other nuclei, but it can have greater intensity; and it must not be connected to the main nucleus (Tolbert et al., 1992; Holland et al., 2007).

There is much uncertainty surrounding studies of pesticide exposure and genotoxic damage, including the reliability of exposure assessment, the power of the studies, the suitability of control groups and the protocols used for determining genotoxicity. In addition, there is by no means agreement as to the significance of an increase in cytogenetic damage for cancer

risk and therefore for the health risks associated with pesticide exposure (Bull et al., 2006).

Therefore, the aim of this study was to evaluate the potential genotoxic damage in fifty agricultural pilots, from Brazil, mainly Central-West and Southeast regions, occupationally exposed to agrochemicals, by means of cytogenetic studies.

Human biological monitoring is a tool of great interest in cancer risk assessment, since it allows estimating genetic risk resulting from environmental exposure to chemicals. The role of the micronucleus test as an intermediate endpoint of carcinogenesis has received much support in the literature, but stronger evidence is available concerning the association between the rate of structural chromosomal aberrations and cancer risk (Bonassi and Au, 2002; Costa et al., 2006).

There are other nuclear anomalies besides micronuclei in the exfoliated cells of the oral mucosa. These nuclear anomalies are binucleated cells, karyorrhexis and broken eggs. They are related to cell death, cell injuries or mistakes during cell division. The frequencies of these anomalies can be evidence of a cytotoxic effect, increasing the specificity of the test, where these anomalies are considered important markers of the process of malignant transformation (Fenech et al., 2007).

MATERIAL AND METHODS

The exposed group (EG) was made up of 50 male agricultural pilots, occupationally exposed to agrochemicals, and the control group consisted of 17 healthy individuals, randomly selected from the Goiania-GO population, with small differences in sex, age and lifestyle, such as smoking and alcohol consumption.

Before collecting the exfoliated oral epithelial cells, the subjects had to rinse their mouths with water to eliminate the presence of food remains and to diminish the concentration of bacteria on the slides. The mucosa was scraped and the cells were spread on slides previously cleaned with absolute alcohol. The slides were dried at room temperature and then, fixed in ethanol for approximately 15 min.

Next, the slides were submitted to acid hydrolysis using 10% HCl (20 mL concentrated HCL in 120 mL distilled water) for 2 min at room temperature. Afterwards, the slides were placed in a water bath at 60°C for 6 min and finally for 2 min at room temperature.

The slides were stained by the Feulgen method and were left in a basic fuchsin solution for 15 min in the dark. Next, the slides were slightly rinsed in water to remove the excess stain and finally counterstained in fast green for 10 min and rinsed in 70% ethanol. To test for other examples of cytogenetic damage, the slides were processed in the same manner.

A thousand cells of the oral mucosa were counted in each case, using a light microscope (400X). The micronuclei were identified by the criteria established by Tolbert et al. (1992). We used the t-test and the Kruskal-Wallis test to evaluate and compare the micronucleus frequencies in the exposed and control groups. We also used the odds ratio to determine the relative risk associated with smoking, alcohol consumption and micronucleus frequencies. All analyses were performed with BioEstat 3.0® (Ayres et al., 2003).

This study was approved by the Ethics Committee of the Fundação Oswaldo Cruz (FIOCRUZ/RJ). All the samples were obtained from the volunteers after obtaining written informed consent. When the samples were collected, all the Brazilian pilots answered a questionnaire regarding information about lifestyle habits, as well as a brief occupational and family history.

RESULTS

The results of the exposed and control groups, taking into consideration age, lifestyle habits, such as smoking and alcohol consumption, the frequency of micronuclei and other nuclear alterations are listed in Table 1. The mean age of the control group was 37 years old (±10), and the mean age of the exposed group was 41.9 years old (±10.6).

The micronucleus frequency observed in the control group was 0.03 and in the exposed group 0.09, indicating a 3-fold increase (P = 0.024). The binucleated cells showed a frequency of 0.03 and 0.08 in the control group and in the exposed group, respectively, i.e., a 2.5-fold increase (P = 0.024). The *t*-test and the Kruskall-Wallis test showed a statistically significant difference (P < 0.05) related to the frequencies of micronuclei and binucleated cells.

Table 1. Mean age, cytogenetic analysis, alcohol consumption and cigarette smoking status of control and exposed groups.

	Age ± SD	%					
		*f*MN	*f*BN	*f*BE	*f*CA	Alcohol consumption	Cigarette smoking
CG N = 17	37.0 ± 10.0	0.03	0.03	0.006	0.02	75	25
EG N = 50	41.9 ± 10.6	0.09	0.08	0.015	0.02	90	15

CG = control group; GE = exposed group; N = sample size; SD = standard deviation; *f*MN = micronucleus frequency; *f*BN = binucleated frequency; *f*BE= broken eggs frequency; *f*CA = karyorrhexis frequency.

In relation to the broken eggs, we observed a frequency of 0.015 and 0.006 in the exposed and control groups, respectively, demonstrating a 2.7-fold increase (P = 0.21).The frequency of karyorrhexis was 0.02 in both groups.

Features of both the control and exposed groups are shown in Table 2. The odds ratio revealed an increase in MN in individuals who consumed alcohol, indicating that they had a 3 times increase in the frequency of micronuclei, when compared to individuals who did not drink. For those who smoked, no difference was found with regard to relative risk.

Table 2. General characteristics of the exposed and control groups.

Characteristics	Exposed group (N = 50)	Control group (N = 17)
Smoking habit	8 (16%)	4 (23%)
Non-smokers	40 (80%)	12 (71%)
No information on smoking status	2 (4%)	1 (6%)
Alcohol consumption		
Alcohol drinkers	43 (86%)	12 (71%)
Not alcohol drinkers	5 (10%)	1 (6%)
Not information on alcohol drinking	2 (4%)	4 (23%)

DISCUSSION

The pilots who participated in our study had inhaled agrochemicals for more than ten years without personal protection equipment, but unfortunately, we did not obtain the list of the pesticides to which they had been exposed, which might have allowed us to associate micro-

nucleus frequency with the types of agrochemicals.

The use of agrochemicals has grown quickly in developing countries, but there is no control in the use of personal protection equipment. Unfortunately, there is no monitoring during the occupational exposure to such agents. The agricultural pilots in the study were often occupationally exposed to agrochemicals, and it is believed that these agents showed cytotoxic and mutagenic properties.

Balmain et al. (2003), in a study of professionals using chemicals to control crop pests, showed the occurrence of chromosomal aberrations. Human exposure to effective mutagenic agents in causing DNA damage constitutes one of the major concerns of public health services, and it is important to evaluate chromosomal damage.

The occurrence of micronuclei can be indicative of chromosomal breakage or interference that might have occurred during cell division, fragmentation of the nucleus and formation of extra nuclear structures with small amounts of DNA (da Cruz et al., 1994). When DNA damage cannot be repaired, as a consequence, an increase in genetic instability can occur, causing, for instance, the development of genetic diseases, such as cancer (Balmain et al., 2003).There is clear evidence of a direct relation between the exposure to genotoxic agents, increase in the frequency of micronuclei and an elevated risk of cancer, which depends on the individual´s constitution, as well as habits and lifestyle (Kausar et al., 2009).

An increase in the frequency of micronuclei in the oral mucosa is related to the development of oral carcinoma. Therefore, the determination of micronuclei in exfoliated cells of the oral mucosa is important, because it allows the identification of genetic alterations directly in the target tissue. The excessive consumption of alcohol and tobacco is a risk factor for oral carcinoma and is associated with an increase in micronucleus frequency (Reis et al., 2006).

Age could also influence the frequency of micronuclei, since they can occur due to the formation of acentric fragments caused by DNA breakage, induced by clastogenic agents, resulting in chromosomal delay in anaphase (Maluf, 2004).

In this study, we did not observe a statistically significant difference in mean age between the exposed group and the controls. Therefore, age did not seem to be a risk factor, according to the differences in frequencies of micronuclei observed between the control and exposed groups. In 2003, Ishikawa et al. in analyzing the occurrence of micronuclei in a Japanese population, found an elevated frequency of micronuclei in individuals 40 years old or older.

Lifestyles, such as smoking and alcohol consumption have been described as risk factors that increase DNA damage. However, we did not find an increase in relative risk in smokers. Other studies showed that alcohol facilitates the penetration of carcinogens into the oral mucosa. This fact can be explained by the solubility of some genotoxic agents or by the increase of mucosa's permeability in the presence of alcohol (Reis et al., 2006). In a study of human cells and other mammalian cells, ethanol could induce gene mutations and sister chromatid exchange in lymphocytes (Maffei et al., 2002).

According to Kehdy et al. (2007), smoking did not influence the cytogenetic parameters examined. In the biomonitoring studies of populations occupationally exposed to genotoxic agents, the influence of smoking on micronucleus frequency is still controversial. A possible explanation is that the damage caused by tobacco could induce cell death in culture or delay the cell cycle, making it impossible to carry out the micronucleus test (Bonassi et al., 2003).

Therefore, diverse agrochemicals used to combat pests in agriculture possess some genotoxic effects observed in the oral mucosa of agricultural pilots. Studies that monitor human popu-

lations exposed to pesticides indicate preventive characteristics against diseases, and reinforce a need for the use of personal protection equipment.

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REFERENCES

Ayres M, Ayres Jr M, Ayres DL and Santos AS (2003). BioEstat 3.0: Aplicações Estatísticas nas Áreas das Ciências Biológicas e Médicas. Sociedade Civil Mamirauá, Belém.

Balmain A, Gray J and Ponder B (2003). The genetics and genomics of cancer. *Nat. Genet.* 33: 238-244.

Bonassi S and Au WW (2002). Biomarkers in molecular epidemiology studies for health risk prediction. *Mutat. Res.* 511: 73-86.

Bonassi S, Neri M, Lando C, Ceppi M, et al. (2003). Effect of smoking habit on the frequency of micronuclei in human lymphocytes: results from the Human MicroNucleus project. *Mutat. Res.* 543: 155-166.

Bull S, Fletcher K, Boobis AR and Battershill JM (2006). Evidence for genotoxicity of pesticides in pesticide applicators: a review. *Mutagenesis* 21: 93-103.

Chen C, Arjomandi M, Qin H, Balmes J, et al. (2006). Cytogenetic damage in buccal epithelia and peripheral lymphocytes of young healthy individuals exposed to ozone. *Mutagenesis* 21: 131-137.

Costa C, Teixeira JP, Silva S, Roma-Torres J, et al. (2006). Cytogenetic and molecular biomonitoring of a Portuguese population exposed to pesticides. *Mutagenesis* 21: 343-350.

da Cruz AD, McArthur AG, Silva CC, Curado MP, et al. (1994). Human micronucleus counts are correlated with age, smoking, and cesium-137 dose in the Goiania (Brazil) radiological accident. *Mutat. Res.* 313: 57-68.

Dietz J, Diehl AS, Prolla JC, Furtado CD, et al. (2000). Pesquisa de micronúcleos na mucosa esofágica e sua relação com fatores de risco ao câncer de esôfago. *Rev. Assoc. Med. Bras.* 46: 207-211.

Fenech M, Bolognesi C, Kirsch-Volders M, Bonassi S, et al. (2007). Harmonisation of the micronucleus assay in human buccal cells-a human micronucleus (HUMN) project (www.humn.org) initiative commencing in 2007. *Mutagenesis* 22: 3-4.

Holland N, Harmatz P, Golden D, Hubbard A, et al. (2007). Cytogenetic damage in blood lymphocytes and exfoliated epithelial cells of children with inflammatory bowel disease. *Pediatr. Res.* 61: 209-214.

Ishikawa H, Tian Y and Yamauchi T (2003). Influence of gender, age and lifestyle factors on micronuclei frequency in healthy Japanese populations. *J. Occup. Health* 45: 179-181.

Kausar A, Giri S, Mazumdar M, Giri A, et al. (2009). Micronucleus and other nuclear abnormalities among betel quid chewers with or without *sadagura*, a unique smokeless tobacco preparation, in a population from North-East India. *Mutat. Res.* 677: 72-75.

Kehdy FS, Cerqueira EM, Bonjardim MB, Camelo RM, et al. (2007). Study of the cytogenetic effects of occupational exposure to pesticides on sanitation workers in Belo Horizonte, Brazil. *Genet. Mol. Res.* 6: 581-593.

Lucero L, Pastor S, Suarez S, Durban R, et al. (2000). Cytogenetic biomonitoring of Spanish greenhouse workers exposed to pesticides: micronuclei analysis in peripheral blood lymphocytes and buccal epithelial cells. *Mutat. Res.* 464: 255-262.

Maffei F, Forti GC, Castelli E, Stefanini GF, et al. (2002). Biomarkers to assess the genetic damage induced by alcohol abuse in human lymphocytes. *Mutat. Res.* 514: 49-58.

Maluf SW (2004). Monitoring DNA damage following radiation exposure using cytokinesis-block micronucleus method and alkaline single-cell gel electrophoresis. *Clin. Chim. Acta* 347: 15-24.

Reis SR, do Espirito Santo AR, Andrade MG and Sadigursky M (2006). Cytologic alterations in the oral mucosa after chronic exposure to ethanol. *Braz. Oral Res.* 20: 97-102.

Tolbert PE, Shy CM and Allen JW (1992). Micronuclei and other nuclear anomalies in buccal smears: methods development. *Mutat. Res.* 271: 69-77.

Ünyayar S, Çelik A, Çekiç FO and Gözel A (2006). Cadmium-induced genotoxicity, cytotoxicity and lipid peroxidation in *Allium sativum* and *Vicia faba*. *Mutagenesis* 21: 77-81.