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ASSESSMENT OF BUCCAL MUCOSA GENOTOXICITY IN INSECTICIDE-EXPOSED URBAN FUMIGATORS IN CALI, COLOMBIA

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Abstract
Objectives: This study aimed to evaluate cytogenetic damage in the buccal mucosa of non-exposed subjects (N = 33) and insecticide-exposed fumigators (N = 31) in the urban area of Cali, Colombia. **Material and Methods:** Through a questionnaire sociodemographic data, anthropometric measurements, state of health, and lifestyle were collected. Buccal micronucleus cytome (BMCyt) assay was using for evaluate cytogenetic damage. **Results:** The study showed that all fumigators used adequate personal protective equipment (PPE) and had low alcohol consumption. The authors did not find significant differences in BMCyt biomarkers between the groups ($p > 0.05$). Multivariate analysis showed a 13% increase in micronucleus (MN) frequency for every year of increasing age (OR = 1.13, $p = 0.029$), and higher MN with the decrease in daily fruit consumption (OR = 4.71, $p = 0.084$), without statistical significance. **Conclusions:** The results between groups could be related to healthy habits and PPE use among the subjects. Int J Occup Med Environ Health. 2024;37(1):128–37

Key words:
insecticides, occupational exposure, buccal micronucleus cytome assay, DNA damage, biomarker, personal protective equipment

INTRODUCTION

The extensive use of insecticides has generated concern about adverse environmental and human health effects [1,2]. Issues associated with these compounds may be underestimated due to under-registration, handling of these compounds, deficient regulatory and surveillance systems, non-compliance with regulations, and inadequate access to information systems [3]. Although the entire population is exposed to these compounds, the population group at higher risk of suffering adverse effects due to acute and chronic insecticides exposure is the operating personnel in charge of formulating, preparing mixtures, and applying them. According to the International Agency for Research on Cancer (IARC), insecticides such

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as organophosphates are classified as probably carcinogenic (Group 2A), while other insecticides as pyrethroids are not classified regarding carcinogenicity in humans (Group 3). Epidemiological studies have reported that organophosphates exhibit genotoxic, mutagenic, teratogenic, hepatotoxic, and neurotoxic effects; while pyrethroids display high toxicity in fish, *in vitro* genotoxicity, potential *in vivo* carcinogenic effects, endocrine disruptions, and bioaccumulation in human breast milk [2]. Most of the genetic biomonitoring studies in populations exposed to pesticides (fungicides, herbicides, and insecticides) have been performed on agricultural rural workers [4–10]. Some pesticides often used in agriculture or public health protection programs have shown genotoxic effects. However, DNA and cellular damage by insecticides exposure, such as organophosphates and pyrethroids at urban workers, have not been sufficiently studied.

Cali, Colombia, a tropical city, has one of the highest prevalences of vector-borne infectious diseases. Therefore, fumigations to prevent endemic diseases like dengue, zika, and chikungunya is extensive. However, the assessment of potential effects from chronic exposure among operators and fumigators has not been sufficiently investigated to date. The buccal micronucleus cytome (BMCyt) assay has become important biomonitoring tool for assessing cytogenetic damage in different populations and discriminating between DNA damage, cell death, and defects in cytokinesis, which could be associated with an increased risk of developing several kinds of diseases [10]. Therefore, this study evaluated cytogenetic damage in epithelial cells of the buccal mucosa of urban fumigators exposed to insecticides, using the BMCyt assay.

MATERIAL AND METHODS

Study population

A cross-sectional descriptive study was conducted during 2017–2018, in the urban area of the city of Cali, Colombia. A non-probabilistic sampling was conducted. The inclusion criteria were males >18 years of age, apparently healthy, and non-smokers. The subjects who self-reported chronic and infectious diseases, family or personal history of cancer, consumption of antibiotics and other xenobiotics, and exposure to ionizing and non-ionizing radiation, during the 4 months before the biological sample collection, were excluded. The study comprised a total of 64 participants, consisting of 31 fumigators and 33 individuals from the general community, residing in the same geographical area without occupationally exposed to insecticides, environmental pollutants, or genotoxic substances. This sample size has been reported to be sufficient to detect differences in cytogenetic effects between the 2 groups [11].

Subject recruitment

Urban fumigators were recruited from public and private entities of pest and vector control programs. The subjects not occupationally exposed were mostly security and surveillance workers from an educational institution. Before inclusion in the study, the subjects were informed about the aim, methods, and risks, and signed informed consent. A structured questionnaire was applied to obtain sociodemographic data (age, sex, socioeconomic status, and educational level), health status. To determine the consumption of fruits, alcohol, and physical activity a frequency questionnaire designed for this study was applied. For the foods the authors made a list of the main foods that are part of the Colombian diet and a table of frequency of consumption per week of each one of them. The research protocol was approved by the research ethics committee of the Faculty of Health Sciences of the Pontificia Universidad Javeriana Cali, Colombia, Acta No. 07-12-2016, according to the guidelines of the World Health Organization (WHO)[12].

Anthropometric measurements

Body weight was measured by a portable scale (130 kg capacity), and height using non-elastic measurement tape (200 cm). Body mass index (BMI) was calculated using body

weight (kg) by the squared height (m). The BMI was used to classify subjects into underweight (<18.5 kg/m^2), normal weight (18.5–24.9 kg/m^2), overweight (25–30 kg/m^2), or obese (>30 kg/m^2). Waist circumference (WC) was measured by taking as reference the midpoint between the xiphoid process of the thorax and a horizontal line between the iliac crests using a non-elastic measurement tape.

Sample collection and processing

Exfoliated buccal mucosal samples were collected with a sterile cytobrush (Interplast®, Medellín, Colombia), performing twenty circular expansion movements on the buccal mucosa of both cheeks. An alphanumeric code was assigned to each biological sample matching the questionnaire. All samples were collected over 5 months (July–December 2017).

Buccal micronucleus cytome assay

Exfoliated cells were suspended in 5 ml of 0.9% NaCl and stored for 4–5 h at 4°C. Then, cell suspensions were centrifuged (1200 rpm for 10 min at 25°C) and washed 3 times with 0.9% NaCl. In each wash, descending volumes (50 µl, 30 µl, and 20 µl) of dimethyl sulfoxide (D-2650 Sigma, Irvine, UK) were used to disaggregate the cells. In the last wash, the supernatant was removed leaving approx. 0.5 ml. The cell suspensions were spread evenly on 2 slides and dried for 15 min at 25°C. The slides were fixed with cold methanol (80%, v/v) for 15 min, dried, and stained with Schiff's reagent (857343 Sigma) and 0.2% (w/v) light green dye (F7258 Sigma) to dry at 25°C. For long-term storage, slides were mounted with a drop of Permount (SP15500 Fisher, Fair Lawn, USA) and 22 × 22 mm^2) glass coverslips. To avoid bias, the slides were coded by the "masking" method.

The cells were analyzed at a magnification of 1000× using a trinocular optical microscope (BS-2040T, BestScope, Beijing, China), and the images were captured with a digital microscope camera (BLC-250A LCD, BestScope). The criteria followed for MNs and/or NBUDs (DNA damage) and nuclear abnormalities in buccal mucosa were according to Bolognesi et al. [13] and Thomas et al. [14]. The MN are characterized by round or oval shape with the same texture and staining intensity that nucleus and diameter of ranges between one-third and one-sixteenth of the main nucleus (Figure 1). The total number of micronucleus (MN) and nuclear buds (NBUD) per cell was recorded in 2000 differentiated epithelial cells. Pyknotic, karyorrhectic, karyolitic, and condensed chromatin cells (nuclear abnormalities) and binucleated (BN) were recorded in 1000 differentiated cells [14]. Cell counting per slide was randomized and performed by an experienced researcher.

Statistical analysis

The group of fumigators was matched for age (±2 years) and socioeconomic level with the subjects in the non-exposed group. The normal distribution of continuous variables was analyzed using the Kolmogorov-Smirnov test. Differences between groups were analyzed by Student's t-test, Mann-Whitney U test, and χ^2 test, according to the distribution and type of variable. Spearman's correlation was used to find relationships between BMCyt biomarkers, age, and exposure time. A multivariate logistic regression analysis was performed having MN frequency as the dependent variable and BMI, physical activity, fruit consumption frequency, age, and study group (non-exposed and fumigator occupationally exposed to insecticides) as independent variables. A p-value ≤0.05 was considered significant. Analyses were performed with SPSS v. 25 statistical software (SPSS Inc., Chicago, IL, USA).

RESULTS

Age, BMI, and WC did not present significant differences between the study groups (Table 1). Regarding BMI, the subjects were overweight (BMI 27.34±0.52 kg/m^2).

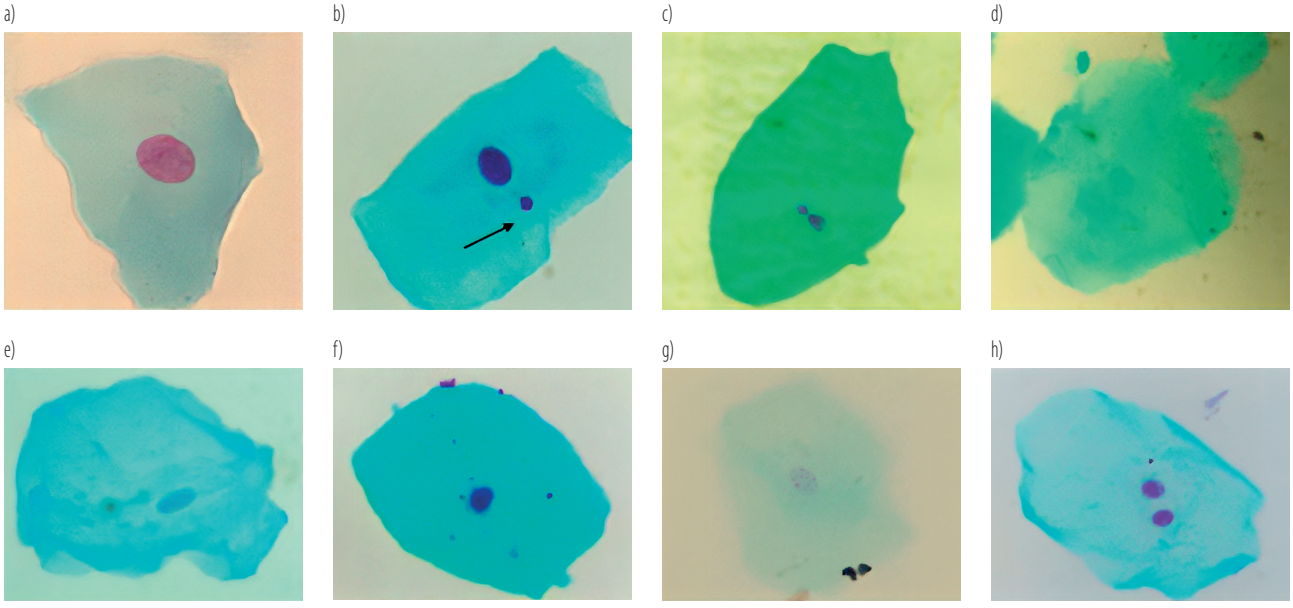

Figure 1. Photographic registry of the genotoxicity and cytotoxicity biomarkers observed using the buccal micronucleus cytome assay: a) differentiated cell, b) cell with micronucleus (MN) (black arrow), c) cell with nuclear bud (NBUD), d) karyolitic cell (KHL), e) cell with condensed chromatin (CC), f) pycnotic cell (PYC), g) karyorrhectic cell (KHC), h) binucleated cell (BN), July 2017–January 2018, Cali, Colombia

Regarding lifestyle, 64.5% of those exposed and 84.8% of non-exposed practiced some type of physical activity; this difference was not significant ($p = 0.06$). Fruit consumption was higher in the non-exposed group ($p = 0.04$), and alcohol consumption was not different between the groups ($p = 0.84$).

The occupational exposure time to insecticides was 5.6 years (range 0.4–22 years) with a daily exposure ≥8 h. All fumigators applied pyrethroids (permethrin and cypermethrin) and 6.4% organophosphates (malathion), and the application method was spraying. All fumigators reported the use of personal protective equipment (PPE) such as masks with filters, impermeable clothes, and rubber boots.

There were no significant differences in BMCyt biomarkers of genotoxic (MN and BN) or cytotoxic (nuclear abnormalities) damage between groups (Table 2). The frequency of MN in the fumigator's group correlated with age ($\rho = 0.495$, $p = 0.005$), this correlation was not found in the non-exposed group ($\rho = 0.100$, $p = 0.579$).

Multivariate logistic regression analysis to study factors associated with MN frequency (Table 3), showed that for each year increase in age, the frequency of MN increases by 13% (OR = 1.13, $p = 0.029$). Fruit consumption from daily to once a week was not associated with MN (OR = 4.71, $p = 0.083$), physical activity and BMI ($p > 0.05$).

DISCUSSION

This study characterized cytogenetic damage in a sample of non-exposed subjects and fumigators exposed to insecticides in the urban area of the city of Cali. The authors did not find a difference in the frequency of biomarkers of genotoxic and cytotoxic damage between the 2 groups. The results are consistent with studies conducted on occupationally exposed subjects to organophosphorus and pyrethroid between 36–44 years old, such as flower growers, applicators, and formulators [10,15,16]. Conversely, the genotoxic potential of pesticides used for pest and vector control has been reported [4,5,7,17,18]

Table 1. Main demographic characteristics and lifestyle of non-exposed subjects and insecticide-exposed fumigators, July 2017–January 2018, Cali, Colombia

Variable	Participants (N = 64)			p
	total	non-exposed (N = 33)	exposed (N = 31)	
Age [years] (M±SE)	36.11±1.11	36.18±1.60	36.03±1.60	0.95
Height [m] (M±SE)	1.69±0.01	1.69±0.01	1.69±0.01	0.77
Weight [kg] (M±SE)	78.23±1.62	78.53±2.44	77.92±2.17	0.85
BMI [kg/m^2] (M±SE)	27.34±0.52	27.53±0.81	27.14±0.64	0.71
Waist circumference [cm] (M±SE)	93.25±1.67	93.23±2.90	93.30±1.66	0.99
Weight status [%]				0.094
<18.5 (underweight)	1	0	1	
18.5–24.9 (normal weight)	19	13	6	
25–30 (overweight)	23	8	15	
>30 (obese)	20	12	8	
Socio-economic level [n (%)]				0.272
1 (low-low)	11 (17.2)	8 (24.2)	3 (9.7)	
2 (low)	28 (43.8)	11 (33.3)	17 (54.8)	
3 (medium-low)	20 (31.3)	11 (33.3)	9 (29)	
4 (medium)	5 (7.8)	3 (9.1)	2 (6.5)	
Education level [n (%)]				0.087
elementary	2 (3.1)	1 (3)	1 (3.2)	
high school	29 (4.53)	15 (45.5)	14 (45.2)	
technician	16 (25)	7 (21.2)	9 (29)	
technologist	8 (12.5)	4 (12.1)	4 (12.9)	
professional	9 (14.1)	6 (18.2)	3 (9.7)	
Alcohol intake frequency [n (%)]				0.84
a few times a week	3 (4.7)	1 (3.0)	2 (6.5)	
a few times a month	15 (23.4)	8 (24.2)	7 (22.6)	
rarely	38 (59.4)	19 (57.6)	19 (61.3)	
never	8 (12.5)	5 (15.2)	3 (9.7)	
Physical activity [n (%)]				0.06
yes	48 (75)	28 (84.8)	20 (64.5)	
no	16 (25)	5 (15.2)	11 (35.5)	
Fruit intake [n (%)]				0.04
every day	11 (17.2)	9 (27.3)	2 (6.5)	
1–3 times a week	27 (42.2)	12 (36.4)	15 (48.4)	
weekly	18 (28.1)	6 (18.2)	12 (38.7)	
monthly	3 (4.7)	3 (9.1)	0 (0)	
rarely	5 (7.8)	3 (9.1)	2 (6.5)	

* Chi-squared test.

Table 2. Genotoxicity and cytotoxicity biomarkers frequency determined by buccal micronucleus cytome (BMCyt) assay of non-exposed subjects and insecticide-exposed fumigators, July 2017–January 2018, Cali, Colombia

Biomarker	Participants (N = 64)		p*
	non-exposed (N = 33)	exposed (N = 31)	
Micronuclei[a] (M±SE)	0.24±0.11	0.13±0.06	0.73
Nuclear bud[a] (M±SE)	1.36±0.23	1.39±0.19	0.73
Pycnotic[b] (M±SE)	16.21±2.82	14.52±1.83	0.94
Karyorrhectic[b] (M±SE)	18.64±3.82	14.58±2.36	0.70
Condensed chromatin[b] (M±SE)	44.55±4.03	38.13±4.12	0.15
Karyolitic[b] (M±SE)	13.97±1.97	16.13±2.39	0.56
Binucleated[b] (M±SE)	1.15±0.22	1.71±0.30	0.13

* Significance between groups. Mann-Whitney U test.
[a] Registered in 2000 differentiated epithelial cells.
[b] Registered in 1000 differentiated epithelial cells.

Table 3. Odds ratios and 95% confidence interval (CI) of micronucleus frequency in exfoliated buccal cells of non-exposed subjects and insecticide-exposed fumigators, July 2017–January 2018, Cali, Colombia

Variable	β	OR	95% CI	p
Age	0.12	1.13	1.01–1.26	0.029
BMI (normal/altered)	0.83	2.29	0.23–23.18	0.483
Physical activity (yes/no)	–041	0.66	0.10–4.34	0.670
Fruit intake (once a week/every day)	–1.55	4.71	0.81–27.44	0.084
Group (non-exposed/exposed)	–0.52	0.60	0.12–3.04	0.534

In this study, pyrethroids and organophosphates were the most often used insecticides by urban fumigators. Organophosphates have genotoxic effects, and both occupational and non-occupational exposures have been associated with adverse human health outcomes [1,19]. However, pyrethroid studies have shown results inconsistent concerning genotoxic effects in biomonitoring studies and have been suggested that pyrethroid exposures primarily affect the male reproductive system [2]. These compounds have a relative selectivity (so their toxicity is low on non-target organisms) and low persistence in the environment. Additionally, pyrethroids are rapidly metabolized by hydrolytic cleavage of ester bonds, followed by oxidation and glucuronidation to produce 3-phenoxybenzoic acid (3-PBA) and cis- or trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane carboxylic acid (cis- or trans-DCCA) in individuals exposed to permethrin and cypermethrin, respectively [20]. The rapid metabolism and elimination (between 4–12 h) of these pyrethroids could explain the reduced cytogenetic effect in the subject studied. Also, the mean time of exposure to insecticides was 5.62 years in the fumigators, which is lower than reported by Lamadrid et al. [16], Wilhelm et al. [6], and Remor et al. [10] (7.6 years, 9.7 years,

and 25 years, respectively), studies that found no cytogenetic damage or association with the time of exposure.

On the other hand, in the study, all fumigators reported the use of adequate PPE during insecticide application; this factor could reduce exposure to insecticides and their effects. For instance, the lack of use of PPE has been associated with a significant increase of 137% in MN distribution in exfoliated cells of the buccal mucosa when compared to those that used PPE, suggesting that the use of PPE may have an important protective effect against exposure to insecticides [7]. Other studies show an opposite tendency (without statistical significance) on MN frequency and the use of PPE [10].

The exposed group showed a positive association between age and the frequency of MN; this association was not found in the non-exposed group. These differences between the 2 groups could indicate an effect of insecticides in frequency of MN. On the other hand, the association between age and the increase in MN has been reported in age groups >40 years old [11], a cutoff point that is higher than the mean age of the sample, which could explain why the association was not significant in the non-exposed group. In the fumigator group, the positive correlation can be explained by occupational exposure to insecticides contributing to a higher frequency of MN with age. This increase could be explained by the accumulation of DNA lesions, due to the progressive deterioration of genome repair capacity and an increase in free radicals related to aging. Xotlanihua-Gervacio et al. [21], reported that the frequency of MN increases in individuals >35 years old exposed to pesticides, a finding similar to this study. Rohr et al. [22] found age-dependent cytogenetic damage in buccal cells with the BMCyt parameters. Higher genotoxic damage with age can be explained by the accumulation of DNA lesions, due to the progressive deterioration of the genome repair ability and the increase of free radicals related to aging, proving age as an important confounding factor.

Related to lifestyle factors, the subjects selected for this study were non-smokers and the majority (approx. 62%) reported occasional or null alcohol consumption. The exclusion of smokers eliminated one of the possible confounding factors associated with MN, due to mutagenic and carcinogenic effects of cigarette compounds. While biological evidence of genotoxicity by alcohol consumption is inconsistent [23,24]. *In vivo* studies performed in mice and rats chronically and sub-chronically [25,26] treated with ethanol showed contrasting results in the frequency of MN, and studies in humans have shown that chronic alcohol consumption or in combination with smoking are related to higher MN frequency [24,27]. In this study, as was mentioned, most of the subjects had low consumption of alcohol without differences between groups. Therefore, this factor could not be associated with DNA damage analyzed.

Diet and nutritional status have a key role in the maintenance of genomic stability; certain vegetable foods contain micronutrients necessary for DNA repair. In this study, the subjects had a high consumption of fruits, which may be a protective factor against genotoxic damage induced by occupational exposure to pesticides, as reported by Martínez-Perafán et al. [28]. The daily consumption of fruits has been associated with a reduction up to 32% in MN frequency compared with a poor consumption [11]. However, the effect of fruit consumption may depend on the number and size of the portion ingested, as well as the type of fruit consumed.

Considering that both age and sex are internal confounding factors when DNA damage is evaluated [21], one strength of this study was the match exposed with non-exposed subjects by age (±2 years), this ensures similarity in this variable for analysis. Some authors affirm that the MN biomarker is influenced by sex. The males could have less MN than females because inactive X chromosome in females induces higher micronucleation [29]. Since in the study the population recruited was males,

this confounding factor did not affect this study results. In addition, the Feulgen Fast green staining technique used avoids the registration of cell bodies or debris resulting from sample processing that could be falsely registered as MN.

For future studies, the authors recommend a larger sample size with genetic biomonitoring studies to identify risk and protective factors, which contribute to the implementation of occupational epidemiological surveillance programs and the use of exposure, effect, and susceptibility biomarkers, to match with national and global occupational cancer control plans [30]. The purpose of these plans is to strengthen the prevention of occupational risks through the promotion of health at work, by methods for the identification of carcinogenic agents with markers of biological effect in exposed populations. The authors also recommended extending the study to other populations susceptible to cytogenetic damage because of environmental exposure to insecticides, such as children, pregnant women, and the elderly. Finally, according to Valencia-Quintana et al. [8] the authors also suggest the use of combined biochemical and cytogenetic techniques to assess the risk of pesticide exposure in urban workers.

CONCLUSIONS

In conclusion, the results suggest that the use of PPE in the workplace and the adoption of healthy self-care habits (daily fruit consumption, low alcohol consumption, and not smoking) in urban fumigators could attenuate the cytogenetic effects of insecticides.

Author contributions

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