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Cytogenetic damage in peripheral blood lymphocytes of children exposed to pesticides in agricultural areas of the department of Cordoba, Colombia

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ABSTRACT

Pesticides offer benefits, like optimization of agricultural production and disease control; however, these toxic substances can contaminate the environment and pose risks to human health. The aim of this study was to assess pesticide exposure and frequency of cytogenetic damage in infant populations in agricultural areas of the department of Córdoba, Colombia. Urine and peripheral blood samples were taken from children living in the villages of La Ceibita (municipality of Cereté), Cabuya (municipality of San Carlos), Aguas Negras (municipality of Montería), Pelayito (municipality of San Pelayo), and the city of Monteria (control group). The work evaluated biomarkers of exposure to pesticides (atrazine urinary concentrations (ATZ) and its metabolites) and biomarkers of cytogenetic damage (micronucleus frequency (MN), nuclear buds, and apoptotic cells in peripheral blood lymphocytes). Measurable ATZ concentrations and/or its metabolites were recorded in the Pelayito, Aguas Negras, and Cabuya zones, which had higher MN frequencies, nuclear buds, and apoptotic cells than the control. Infant exposure to one of the more-often used pesticides in the agricultural areas evaluated and an increasing trend in the frequency of markers of cytogenetic damage in the groups of the agricultural areas, as compared to the control group, were evident.

1. Introduction

Pesticide manufacture uses around 1000 active ingredients, sprayed mainly in rural agricultural areas to reduce and/or eliminate populations of insects, fungi, and weeds, among other things, for crop protection [1]. Although these substances offer benefits, such as optimization of agricultural production and disease control, their extensive use tends to pollute the air, water, soil, and food [2], representing risks to human health and the environment [3].

Adverse effects on human health caused by exposure to pesticides include discomfort, permanent damage, and irreversible changes in the nervous, reproductive, and hormonal systems among others. Additionally, DNA alterations that can be transmitted or manifested in subsequent generations, some of which have been classified as carcinogenic and probable or possible carcinogenic for humans, and which may be lethal in cases of severe poisoning [4–7]. The association between mutagenic, genotoxic, and carcinogenic activities in humans and exposure to pesticides has been widely recognized [8,9]. Using biomarkers of cytogenetic damage, such as micronucleus testing (MN), offers evidence of early DNA damage; it has been used to evaluate the effects of these substances on the genetic material of adult and infant populations with occupational or environmental exposure [3,10–12].

These health changes caused by pesticides can occur more easily in infant populations because of their vulnerable stage of development, which makes them more sensitive to the effects of these pollutants [13]. Children may respond differently than adults in the detoxification process, DNA repair, and cell proliferation [14], given that their metabolism is more rapid than that of adults [15]. In this sense, it is more likely that early DNA alterations not adequately repaired in children can lead to a faster development of major alterations in health.

In Colombia, despite being a country with a high use of pesticides for agriculture [16], no studies exist on pesticide exposure in children or evaluations of cytogenetic alterations in any class of cells in infant populations in agricultural areas potentially exposed to pesticides. However, some studies have evaluated the frequency of chromosome aberrations, MN, deficiencies in lymphocyte DNA repair activity [17], acetylcholinesterase activity [18,19], and genotoxic risk with a lymphocyte micronucleus assay [20] in adult populations with

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occupational exposure to pesticides. The aim of this study was to evaluate pesticide exposure and frequency of cytogenetic damage in infant populations in agricultural areas of the department of Córdoba, Colombia. The study used as exposure biomarkers, urinary concentrations of one of the most-often used pesticides in the study areas (atrazine and its metabolites) and as biomarkers of cytogenetic damage, the MN frequency, nuclear buds, and apoptotic cells in peripheral blood lymphocytes.

2. Materials and methods

2.1. Study area

The study was conducted in rural farming areas of the middle basin of the Sinú River in the department of Córdoba, Colombia (Ceibita village – municipality of Cereté: 8°49′35" N – 75°39′03" W, Cabuya – municipality of San Carlos: 8°46′18" N – 75°43′53" W, Aguas Negras – municipality of Montería: 8°48′44.96" N – 75°49′1.81" W, Pelayito – municipality of San Pelayo: 8°56′20.8" N – 75°49′5.74" W, and the city of Monteria as the control group: 08°45′27″ N – 75°53′08″ W). The middle basin of the Sinú River is between 10 and 20 m a s l., with an average annual temperature of 29 °C, annual rainfall of 1200 mm, and 85% relative humidity [21]. Traditionally, much of this region has been dedicated to agriculture because of the low concentrations of salts in the Sinú River, a characteristic that provides soil conditions suitable for growing a wide variety of crops (corn, rice, cotton, and cassava, among others), with corn and cotton in the main study areas [22]. Although no official statistics or records are available for the quantities of pesticides used in this region, it is clear that commercial agriculture has evolved and expanded over the decades, which has used and continues to consume large amounts of these substances for crop protection.

2.2. Type of study and target population

An exploratory cross-sectional study was conducted in which the frequency of cytogenetic damage markers in peripheral blood lymphocytes (micronucleus assay) and the presence or absence of markers of exposure (metabolites) to pesticides was evaluated in urine samples from children. The study included children between 5 and 15 years of age from both genders (male and female), with a minimum stay in the study area of three years. To reduce the likelihood of bias in the interpretation of the results, children with birth defects, chronic degenerative diseases, requiring permanent medication, who have been exposed to radiation (X-ray examinations) within the previous six months and those who that manifested having had the habit of smoking or consuming liquor were excluded from the study.

In each area or study population, an enrollment list (with the parents) of 20 children was made of which a smaller number in each population (Aguas Negras = 17 children, Cabuya = 8 children, Pelayito = 12 children, Ceibita = 13 children, and Control group = 13 children) finally participated in the study, given that on the sampling day some children did not allow to be sampled, or their parents or legal guardians (an adult relative) were unable to attend. All of the participants were selected through non-probability intentional sampling.

2.3. Collection of information

A survey was conducted with the parents or legal guardians of children to collect demographic information, regarding exposure characteristics and health status of the children. From this information, the study participants were selected based on the criteria described in the previous section.

2.4. Ethical aspects

The Ethics Committee of the Faculty of Health Sciences at Universidad de Córdoba, Colombia approved the study. According to Resolution No. 8430 of 1993 from the Colombian Ministry of Health and Social Protection [23], prior to sampling, the parents or legal guardians of each child were asked to sign an informed consent form that was explained clearly and completely, indicating the purpose, scope, and limitations of the study and the associated risks. Signing this document meant the parents authorized participation by the children.

2.5. Sampling

Urine samples were collected in sterile plastic containers early in the day (between 6:00 am and 7:00 am) by the participants themselves with help from their parents, who were provided instructions for labeling and cold storage. Peripheral blood samples were taken between 7:00 am and 9:00 am through venipuncture on the forearm with heparinized vacutainer tubes (4 mL). Both samples were stored and transported for 1–1.5 h in controlled-temperature containers (20–22 °C) for the blood samples and plenty of ice for the urine samples, to the Toxicology and Environmental Management Laboratory at Universidad de Córdoba for immediate processing.

2.6. Biomarkers of exposure to pesticides

Urinary concentrations of one of the most-often used pesticides in the study areas, atrazine (ATZ) and its metabolites atrazine desisopropyl (ADI) and atrazine desethyl-desisopropyl (ADDI), were used as biomarkers of exposure to pesticides. The analysis was performed with gas chromatography-mass spectrometry (GC–MS Trace 1310 Thermo Scientific gas chromatograph and ISQ Thermo Scientific Mass Spectrometer) after liquid–liquid extraction with ethyl acetate and diethyl ether, and clean-up of the extracts (Florisil and sodium sulfate), under the following chromatography conditions: injection volume = 1 μL, detector temperature = 285 °C, DB5MS capillary column: 30 m x 0.2 mm x ID 0.20 μm and Helium as carrier gas [24]. The limits of detection (LD in ppb) and quantification (LC in ppb) of the method were ATZ: LD = 0.451; LC = 0.455; ADI: LD = 3.194; LC = 3.200; ADDI: LD = 7.123; and LC = 7.130.

2.7. Biomarkers of cytogenetic damage

Frequency of MN, nuclear buds, and apoptotic cells in peripheral blood lymphocytes was used as indicators of cytogenetic damage, assessed with micronucleus assay with block cytokinesis with cytochalasin B, according to the protocol described by [25]. The lymphocytes were separated from the whole blood sample using the Ficoll-1077 technique (Histopaque-1077™), per manufacturer's instructions. The lymphocyte culture was done in RPMI 160, supplemented with fetal bovine serum, L-Glutamine, antibiotics, and phytohemagglutinin for 72 h at 37 °C and 5% CO_2, adding cytochalasin B at 44 h. At the end of cultures, preparations were made on microscope slides with a cytological centrifuge (Cellspin Hanil) at 500 rpm for 5 min, which were dried at room temperature, fixed with 80% cold methanol (v/v), and colored with a 10% Giemsa solution (v/v) in a Sörensen buffer for 15 min. The frequency of DNA damage biomarkers (MN and nuclear buds) was scored in 2000 binucleate cells (1000 duplicated) and the frequency of apoptotic cells was analyzed in 1000 viable cells. Frequency of all alteration was expressed as frequency of alterations for 1000 cells scored and the analyses were performed in an optical microscope (Olympus CX41) at 1000X magnification.

2.8. Statistical analysis

Kruskal-Wallis (KW) analysis and Dunn comparison of average-range test were used to assess differences in the frequency of markers of cytogenetic damage (MN, nuclear buds and apoptotic cells) and socio-demographic variables among the study groups. A Spearman