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Assessment of exposure to pesticides in rural workers in southern of Minas Gerais, Brazil

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ABSTRACT

The aim of the study was to assess of occupational exposure to pesticides in rural workers using genotoxicity test, bioindicators and clinical evaluation. Blood, urine and buccal samples from persons, rural workers exposed to a complex mixture of pesticides with organophosphates (n = 94) and without organophosphates (n = 94) were collected to compare the activities of cholinesterases, the levels of urinary dialkyl phosphates, genotoxicity data, from a cytome assay. Biomarkers were analysed by traditional/published methods Control group consisted of 50 other persons, non- occupationally exposed to pesticides from the city of Alfenas, Minas Gerais, Brazil. All subjects underwent a clinical evaluation. In the group exposed to organophosphates, the activity of acetylcholinesterase, butyrylcholinesterase and total cholinesterase was lower by 63.8%, 12.8%, and 14.8%, respectively, and 92.6% of the group had dialkyl phosphates present in their urine. The cytome assay was used to measure biomarkers of DNA damage (micronuclei and/or elimination of nuclear material by budding), cytokinetic defects (binucleated cells), and proliferative potential (basal cell) and/or cell death (condensed chromatin, karyorrhectic, pyknotic, and karyolytic cells). The group exposed to organophosphates showed significant changes in all these parameters compared to the control group and showed significant changes in budding, condensed chromatin and karyolytic cells compared with the group non-exposed to organophosphates. Data from the clinical evaluation showed significant changes in the central nervous, respiratory and auditory systems. The studied biomarkers are able to distinguish occupational and environmental exposure to pesticides and the data showed hazardous exposure to organophosphates and afforded valuable data to estimate the risk to cancer development.

1. Introduction

The indiscriminate use of chemicals in agriculture has increased significantly, increasing the risk of occupational exposure and toxication. In tropical regions exposure increases due to humidity and high temperatures, which cause these substances to remain in the air because of their association with water molecules and these substances can be reach with others chemicals. The irrational use of the pesticides can be lead to toxic effects, in the humans exposed environmental and occupationally, as these chemicals arenŕt selectivity of the toxicodynamics (Martínez-Valenzuela et al., 2009). Campos et al. (2016) discussed that exposure to pesticides could be related mental disorders.

Rural workers are exposed to a complex mixture of herbicides, fungicides, insecticides, and other pesticides (Benedetti et al., 2013; Campos et al., 2016) and organophosphates represent a class of pesticides with high toxicity and widespread use (Santos et al., 2012; Lionetto et al., 2013). The effects of long-term exposure to low doses are often difficult to evaluate, by the absence of clinical manifestation

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(Benedetti et al., 2013). Exposure to these complex mixtures is a high health risk for rural workers, as acute and chronic forms of exposure may lead to irreversible damage and death (Hernández et al., 2005; Martínez-Valenzuela et al., 2009; Benedetti et al., 2013). As widely described damage on the nervous and on the respiratory systems and the reproductive organs, as well as dysfunction of the immunological and endocrine systems and mutagenicity and carcinogenicity, are associated to pesticides exposure (Martínez-Valenzuela et al., 2009).

Classical biological indicators, the determination of cholinesterase activity from blood and urinary dialkyl phosphates (DAPs) are recommended to evaluate the exposure and the effects of organophosphates (Santos et al., 2012). Additionally, human buccal micronucleus (MN) represents an alternative as biomarker to assessing the exposure and the risk to genotoxic pesticides since some of these compounds are considered possible carcinogens, as initiators of this process, and can lead to a higher incidence of diseases and malformations, as a consequence of their genotoxicity (Benedetti et al., 2013). The biomonitoring of the workers exposed to pesticides have shown MN (Martínez-Valenzuela et al., 2009). The formation of MN is therefore induced by substances that cause breakage of chromosomes (clastogens) as well as by agents which affect the spindle apparatus (aneugens) (Benedetti et al., 2013; Diler and Celik, 2011; Kirsch-Volders et al., 2014; León-Mejía et al., 2014; Rohr et al., 2013).

Differences in the periods, levels of exposure, type of pesticides, mixtures composition, and geographical and meteorological characteristics of the area and differences mainly in the population of rural workers and the population that lives near the agricultural area, difficult to compare the published studies (Martínez-Valenzuela et al., 2009). Therefore, the objective of this study was to assess of occupational exposure to pesticides in rural workers using genotoxicity test, bioindicators and clinical evaluation in Brazilian people exposed to organophosphate pesticides compared with those exposed to non-organophosphate pesticides and those not occupationally exposed to these substances using cholinesterases levels, urinary DAPs, buccal MN cytome assay and clinical evaluation.

2. Materials and methods

2.1. Study design

The study was conducted in the southern region of Minas Gerais between February and November 2014 and January and June 2015, which were periods of intensive pesticide use. Two types of agricultural workers were recruited: those occupationally exposed to complex mixtures without organophosphates (field control, n = 94) and those occupationally exposed to complex mixtures with organophosphates (n = 94). In addition to spraying plantations, agricultural workers also prepare the pesticide mixtures and refill the tanks. The following criteria and characteristics were adopted for inclusion and/or exclusion of workers in the referred groups: persons exposed to organophosphorus insecticides within 60 days prior to the survey and second group, subjects not exposed for at least 1 year to organophosphate insecticides. In the complex mixture of pesticides which are exposed are all workers including triazoles class of compounds, dithiocarbamates, glyphosate, carbamates, pyrethroids and neonicotinoids, among others. The control group (n = 50) comprised workers not occupationally exposed to pesticides or any other suspected genotoxic agents, such as X-rays, who were living in an urban area and had professions such as teacher, laboratory assistant or domestic, among others from the city of Alfenas, Minas Gerais (MG), Brazil. This study was approved by the Ethics Committee of the Federal University of Alfenas-MG-Brazil (n° 415.856). Informed consent was obtained from each volunteer. In addition, each study volunteer completed a questionnaire containing information about demographic data (age, gender, etc.), lifestyle (smoking, coffee and alcohol consumption,diet, etc.) and occupation (number of working hours per day, number of days worked per year, and personal protective equipment). The clinical evaluation was performed using another standardized questionnaire. The instrument used for collection of epidemiological and clinical data of rural workers, was obtained from the University of Campinas (Unicamp) and modified by researchers in which it was carried out the process of refinement by judges and also a pilot test with 50 rural workers. The application of the instrument and clinical evaluation were performed by doctors and a team of trained applicators for this purpose. All samples and data were subjected to a blinded analysis.

The heparinized blood was centrifuged at 2000 rpm for 5 min to obtain plasma samples. The anticoagulant heparin is the only anticoagulant that does not interfere with the activity of the cholinesterase enzymes. Blood and buccal samples were transported at 8 °C and stored at 4 °C. The urine samples were collected and stored at −80 °C until analysis.

2.2. Activity of acetylcholinesterase, butyrylcholinesterase and total cholinesterase

The activities of erythrocyte acetylcholinesterase (AChE), butyrylcholinesterase and total cholinesterase were determined according to the kinetic method of Ellman et al. (1961). The absorbance of the reaction was measured at 430 nm using a Shimadzu spectrophotometer UV 180. The results were compared to reference values established from a set of 100 non-exposed persons from the city of Alfenas, Minas Gerais, Brazil, as a regional reference value.

2.3. Determination of DAPs

The extraction of urinary analytes was performed by a solid phase extraction ion exchange interaction using a Supelco LC-SAX SPE cartridge for diethyl thiophosphate (DETP) and diethyl dithiophosphate (DEDTP) analytes. Briefly, the urine was acidified with 0.1 M hydrochloric acid to pH 4. The conditioning was performed on the cartridge with 2 mL of methanol, 2 mL of Milli-Q water, and 2 mL of acetonitrile. Following this procedure, the charging of the cartridge was performed with 2 mL of urine. Washing was performed with 2 mL of *n*-hexane, and elution was performed with 3 mL of a mixture of methanol and 1% sodium chloride in a ratio of 2:1. These steps were performed in a centrifuge. The eluate was evaporated in a water bath at 55 °C under a nitrogen stream. The ensuing derivatization was performed with 2,3,4,5,6-pentafluorobenzyl bromide (PFBBr), as described by Silvério et al. (2015). The derivative was evaporated in a nitrogen current and was resuspended in 75 µL of toluene and analysed by gas chromatography and mass spectrometry (GC/MS) under the conditions specified by Santos et al. (2012). The analytes DETP and DEDTP were identified by *m/z* 350 and 366 and a retention time of 4.0 and 4.3 min, respectively. Validation was performed according to Resolution RDC N. 27 of May 17, 2012, by ANVISA (2012).

2.4. Buccal MN cytome assay

Buccal mucosa cells were obtained by gently rubbing the inside of the cheeks (right and left side) with a cytobrush, which was then immersed in 5 mL of cold saline (0.9% [w/v] aqueous NaCl) in a Falcon tube and transported under refrigeration to the laboratory. Buccal MN cytome assay was performed as described by Benedetti et al. (2013).

The slides were stained with 2% acridine orange for 5 min, rinsed in distilled water for 2 min, and covered with a cover slip. The reading was performed using a fluorescence microscope (Nikon® Eclipse 50i) with a blue filter and a magnification of 1000. The exfoliated buccal cells were scored as per Thomas et al. (2008) and Kausar et al. (2014). The normal and abnormal cells were distinguished on the basis of nuclear morphology, which were scored as indicator of DNA damage. For each individual, the frequency of the various cell types in the assay is represented as the number of cells in 2000, as suggested by Benedetti