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RESEARCH ARTICLE

Prevalence of chromosomal aberrations in Argentinean agricultural workers

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Abstract Little is known about biosecurity measures and toxic effects during pesticide application in the province of Jujuy, Argentina, particularly concerning the protective measures and mixture of pesticides used by rural workers. We carried out an observational study of agricultural workers from Jujuy (76 exposed subjects and 53 controls) to investigate the prevalence of chromosomal aberrations (CAs) in human lymphocytes as well as the activity level of acetylcholinesterase (AChE) in red blood cell erythrocytes. Whole blood samples (5 mL) were collected in heparinized Vacutainer tubes for cytogenetic analysis and erythrocyte cholinesterase activity determination according to Ellman's method. Cytogenetic results showed a significant CA increase in pesticide-exposed individuals as compared with controls (4.20 ± 0.15 vs. 1.00 ± 0.05, respectively; $p < 0.001$), suggesting that pesticides are clastogenic agents causing DNA damage. Erythrocyte cholinesterase activity was significantly lower in exposed individuals, evidencing the possible occurrence of perturbations in blood as well as neurotoxicity in pesticide sprayers. These results suggest the need for periodic biomonitoring of these biomarkers together with education and training of occupational workers for the safe application of potentially harmful pesticides.

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Introduction

Insecticides are still widely used as pest control worldwide because of their economical value. The intentional application of these chemicals for agricultural and domestic purposes results in both direct and indirect human exposure (Burns et al. 2013). Long-term exposure to large amounts of pesticides by agricultural workers is a matter of concern due to the in vitro and in vivo mutagenic effects of several pesticides and the short-/long-term effects they may induce, such as cancer and congenital malformations (Bolognesi 2003; Beane Freeman et al. 2005; Cocco et al. 2005; Pastor et al. 2002; Lee et al. 2004). Although million cases of pesticide poisonings are documented every year around the world, data of their cytogenetic effects on occupationally exposed individuals are limited (Sharma and Sharma 2012; Sharma et al. 2012a), particularly in developing countries where pesticides have been widely used over the years.

Occupational exposure to pesticides is associated with an increased risk or incidence of different types of carcinomas, such as non-Hodgkin's lymphoma (Orsi et al. 2009; Bertrand et al. 2010; Beane Freeman et al. 2005). Genotoxicity biomarker studies in exposed individuals have found genotoxic effects using cytogenetic endpoints, namely, chromosomal aberrations (CAs) and micronuclei (MN) frequency (Bolognesi et al. 1993; da Silva et al. 2008; Fairbairn et al. 1995; Falck et al. 1999; Grover et al. 2003; Heuser et al. 2007; Moller et al. 2000).

In Argentina, few studies have evaluated genotoxic effects in human populations exposed to pesticides. In the province of Buenos Aires, an increased frequency of total CAs (sister chromatid exchanges and dicentric and ring chromosomes) in lymphocytes has been reported in greenhouse flower growers exposed to organophosphorus, carbamate, thiocarbamate, and organochlorinated pesticides (Dulout et al. 1985). However, the frequency of chromosomal damage did not increase in greenhouse potted plant workers exposed to similar pesticides, but with a different management of these chemicals (Dulout et al. 1987). More recently, an increased MN frequency in buccal mucosa of children exposed to pesticides was reported in the province of Córdoba (Bernardia et al. 2015).

Blood cholinesterases (ChEs) have been widely used for monitoring the exposure to organophosphorus and carbamate pesticides. In neural tissues and erythrocytes, ChE are known as true acetylcholinesterases (AChE), whereas those found in blood serum and synthesized by liver have been termed pseudo- or butyrylcholinesterases (BChEs) (Gupta et al. 2015, 2016; Srivastava et al. 2012; Sharma et al. 2012b, Nigg and Knaak 2000) The association between pesticide exposure and symptoms is strong and, in this sense, ChEs are significantly reduced in exposed populations (Abu Mourad 2005; Singh et al. 2007; Ali et al. 2008; Sharma et al. 2013). Therefore, blood AchE and BChE activity measurement is a good biomarker for this kind of exposure. Blood ChE measurement is also useful as a primary biomarker in emergency medicine in cases of poisoning and accidental organophosphate or carbamate exposure (Ng et al. 2009; Simoniello et al. 2010).

The province of Jujuy, located in northwestern Argentina, has an agrofood production structure characterized by the predominance of family farming. Two studies performed by Altamirano et al. (2003, 2004) determined the activity of plasma AChE in adult rural workers and schoolchildren from the Department of El Carmen, Jujuy. The epidemiological data obtained reflected that the health of the entire rural population was compromised by contamination with pesticides and that efforts should be increased to intervene risk factors and design training and prevention strategies. Lenys et al. (2003) determined and compared the physical–chemical and cytomorphological characteristics of semen in workers exposed to ChE-inhibiting pesticides and related these findings to total blood ChE levels. The results indicated that ChE-inhibiting pesticides affected some spermatogram variables and, consequently, semen quality.

The aim of the present study was to determine the toxic effect of pesticide exposure on agricultural farmers from the province of Jujuy, Argentina, using the CA test in peripheral blood lymphocytes and measuring biochemical parameters such as AChE activity.

Materials and methods

Study population

The study sample ($n = 129$) of this observational study included rural workers from the Departments of El Carmen, Tilcara, and Ledesma (exposed group, $n = 76$), and from San Salvador de Jujuy, the capital city of the province of Jujuy (control group, $n = 53$), in northwestern Argentina (Fig. 1).

El Carmen is located in the zone of temperate valleys of the province, 23 km far from the capital city. The Department is easy to access from national routes 9 and 66. Due to its ecological characteristics, the area is mainly devoted to snuff cultivation and livestock production and, to a lesser extent, to viticulture and horticulture.

Tilcara belongs to the area of the Humahuaca Ravine; it is located at 2334 m.a.s.l. and 76 km far from the capital city, with easy access by national route 9. The economy of the department is based on farms that supply fruits and vegetables to nearby locations.

Ledesma is southeast of the province, about 65 km from the provincial capital, easily accessible through national route 34.

The three departments are located in the region of Yungas and temperate valleys, where the main activity is agriculture, mainly devoted to growing tomatoes, corn, green beans, eggplant, peppers, cucumbers, and squashes. Most inhabitants are originally from Jujuy, although there is a strong Bolivian immigration. The sociocultural level of the exposed population was very low as compared with the control group, with the consequent inadequate use of pesticides by rural workers. In the three departments, most producers were small producers, some of them organized in farming cooperatives. In general, they mixed different agrochemicals for fast application in their crops and to avoid harvest losses, ignoring handling and preparation instructions and without taking any precaution. In the case of rural workers, only a few were aware of the pesticides they were applying and of the effect on their health; they did not wear any kind of personal protective measures, like gloves, arm protection or face mask while mixing, handling or applying the stock of pesticide formulations. Thus, there was direct exposure through inhalation, skin and eyes, with an average exposure time of 10 years.

All participants were provided with specific written information about the aim of the study and gave their written informed consent. Prior to blood collection, each individual completed an anonymous structural questionnaire specifying sex, age, smoking and dietary habits, alcohol consumption, previous exposure to diagnostic X-rays, use of therapeutic drugs, and work-related exposure to hazardous agents. To ensure the anonymity of the participants, each survey was numbered. Further questions related to farming were asked to individuals in the exposed group, such as pesticide use and duration of exposure. Inclusion criteria were moderate alcohol

Fig. 1 Departments of the Province of Jujuy, Argentina, where the study was conducted

consumption and smoking, not having received X-rays, genotoxic agent exposure, or therapeutic drugs during the past year. Exclusion criteria included participants who did not sign the informed consent prior to the survey, heavy smokers (≥20 cigarettes per day in the last 10 years), consumers of more than 5 glasses of alcohol per day, and individuals exposed to harmful factors other than those included in this study. The information was entered into a specially designed database. The demographic characteristics of the study population are shown in Table 1.

Table 1 Demographic characteristics of the study population

	Control group	Exposed group
Sample size (*n*)	53	76
Age	29.85 ± 10.90	36.27 ± 12.05
Exposure time (years)	–	10.75 ± 5.36
Smoking status (%)		
Nonsmoker	95	80
Smoker	5	20
Alcohol consumption (%)		
Nondrinker	32	32
Drinker	68	68

Age and exposure time are expressed as means ± standard deviation

Blood samples were collected at hospitals from the three Departments where the study was conducted and in the capital city of San Salvador de Jujuy, following the same criteria and considering that controls were not in contact with pesticides. All individual were invited to participate by community health agents.

Cytogenetic analysis

Blood samples (5 mL) were collected in Vacutainer tubes. They were cultured for 52 h in Ham's F10 medium supplemented with phytohemagglutinin at the concentration recommended by the supplier, 60 IU/mL penicillin, 50 μg/mL streptomycin, and 10% fetal bovine serum (*v*/*v*). Two hours before harvesting, 1 μg/mL colchicine was added to the medium. After centrifugation, lymphocytes were resuspended in 5 mL 0.075 M KCl and incubated at 37 °C for 40 min. Fixation was carried out with methanol/acetic acid (3:1) at room temperature. Chromosomal preparations were made by dropping the cell suspension onto a slide and then staining with 5% Giemsa solution. They were blind-counted and scored for blind aberrations on coded slides. A total of 200 metaphases per donor were scored to check the incidence of structural CAs.

Erythrocyte ChE activity determination

Heparinized whole blood samples (5 mL) were provided by the Toxicology Sector of the Central Reference Laboratory, Ministry of Health of the province of Jujuy. They were used to determine erythrocyte cholinesterase activity according to Ellman's method (1961). Red blood cells were washed with normal saline and hemolyzed with distilled water. Analyses were carried out with a rate of 25 μL of hemolysate. The enzyme catalyzes the hydrolysis of acetylcholine and acetylthiocholine to acetate and thiocholine. Thiocholine reacts with 5,5-dithiobis-2-nitrobenzoic to produce 2-nitro-5-mercaptobenzoate, colored compound. The catalytic concentration was determined in a spectrophotometer at 412 nm during 2–3 min. Enzymatic activity was expressed as international units per liter (IU/L).

Statistical analysis

The cytogenetic analysis of data was carried out using Mann–Whitney *U* test. Differences in age, smoking, and alcohol consumption were analyzed using Wilcoxon test, whereas differences in AChE activity were compared using Kruskal–Wallis test. Variables were grouped into the following categories: exposure (control/exposed), sex (female/male), smoking (yes/no), alcohol consumption (yes/no), and age (young/adult/elderly people). Statistical significance was set at $p < 0.05$.

Results

Cytogenetic study

The cytogenetic analysis of the 129 blood samples showed monochromatid and isochromatid breaks, as well as dicentric chromosomes. CAs are presented as the cumulative number of aberrations per 100 metaphases. Taking all types of aberrations together, differences in CA frequency between exposed and control individuals were significant (4.20 ± 0.15 vs. 1.00 ± 0.05 respectively; $p < 0.001$) (Table 2). However, differences between categories in both study groups were not significant, showing that these variables were independent of CA frequency (control vs. exposed: smoking, $p = 0.4169$ vs. 0.2982; alcohol consumption, $p = 0.8777$ vs. 0.2949; sex $p = 0.2636$ vs. 0.1775).

Erythrocyte ChE activity

AChE activity in the exposed group was decreased compared with controls (8854.905 vs. 4647.34 IU/L; $p < 0.0001$). On the other hand, the Kruskal–Wallis test did not show marked differences between categories in the control and the exposed groups (sex, $p = 0.7951$ vs. 0.5493; smoking, $p = 0.0508$ vs. 0.9530; alcohol consumption, $p = 0.6530$ vs. 0.2190), demonstrating that AChE activity was higher in the control group as well as independent of age, sex, smoking habit, and alcohol consumption.

Discussion

The widespread use of pesticides worldwide has become a major environmental health problem, particularly for occupationally exposed subjects who are affected by their potentially hazardous effects. Even though the genotoxic potential of pesticides is low, the genotoxic monitoring of agricultural workers could be useful in estimating the risk of long-term health effects such as cancer and adverse reproductive health outcomes (McCauley et al. 2006).

Free radicals are essential for pesticide and environmental chemical toxicity. Pesticide chemicals can induce oxidative stress (OS), thus generating free radicals and altering antioxidants or the oxygen free radical scavenging (OFR) enzyme system (Agrawal and Sharma 2010; Sharma and Sharma 2012; Jaiswal et al. 2016; Sunil et al. 2014, 2016). In this context, lipid peroxidation could be one of the molecular mechanisms involved in pesticide-induced toxicity (Khrer 1993).

The detrimental effects caused by reactive oxygen species (ROS) occur as a consequence of an imbalance between oxidative and antioxidant indices in an individual due to pesticide-induced toxicity (Zeljezic et al. 2008). SOD activity inhibition may cause an accumulation of superoxide radicals. Catalase activity reduction may lead to an accumulation of peroxide and cause membrane lipid peroxidation via the

Table 2 Prevalence of chromosomal aberrations per 100 cells

	Metaphases[a]	Monchromatid breaks	Isochromatid breaks	Dicentric chromosomes
Control	1.00 ± 0.05	0.10 ± 0.03	0.05 ± 0.02	0.05 ± 0.01
Exposed	4.20 ± 0.15	0.50 ± 0.05	0.10 ± 0.03	2.00 ± 0.11
p value	$p < 0.001$	$p < 0.001$	$p = 0.059$	$p < 0.001$

Results are presented as means ± standard deviation. *p* value <0.05 indicates significant differences between control and exposed groups

[a] Metaphase with at least one chromosomal aberration

Fenton reaction (Fetoui et al. 2008). The reduction in these enzymes indicates the failure of the antioxidant defense system and can induce DNA damage; consequently, CA frequency increases, as was observed in this study.

The comparison of different studies performed to date is hindered by the high number and variety of chemicals generally used. In our study, more than 20 different pesticides could be listed (Table 3). However, it is largely unfeasible to determine the potential effects of any specific pesticide of concern because pesticide products generally comprise a mixture of a different chemical, more than one product is used simultaneously, and varying combinations are applied (Bull et al. 2006).

Although in our study pesticide exposure was significant and we counted with information on the genotoxicity of several of the pesticides used, data concerning the genotoxic effects of complex mixtures are missing. Another explanation for the genotoxic damage observed could be the lack of protective measures taken by the farmers.

AChE activity in occupational workers was also decreased as compared with control subjects, which is in agreement with previously reported studies (Hernandez et al. 2005; Jintana et al. 2009). Pesticides can inhibit AChE activity as they modulate the active site of the enzyme, resulting in an excessive accumulation of acetylcholine at the nerve endings and causing blockade of nerve impulse transmission. Organophosphates (OPs), the esters of phosphoric acid, are a class of irreversible AChE inhibitors (Grupta and Bechan 2016). The cleavage of OP by AChE leaves a phosphoryl group in the esteratic site, which is slow to be hydrolyzed and can bound covalently. Carbamates, esters of N-methyl carbamic acid, are reversible inhibitors of AChE that hydrolyze in hours and occupy the esteratic site for short periods of time.

Table 3 Pesticides used by exposed individuals

Pesticides	Compound	Chemical class
Fungicides	Benomyl	Benzimidazole
	Maneb	Carbamate
	Carbendazim	Benzimidazole
	Mancozeb	Dithiocarbamate
	Captan	Thiophthalimide
	Zineb	Imidazole
	Fosetyl-aluminum	Phosphoric acid salt
	Carbendazim	Benzimidazole
	Folicur	Thiazole
Insecticides	Deltamethrin	Pyrethroid
	Methyl parathion	Organophosphorate
	Lambda-cyhalothrin	Pyrethroid
	Methidathion	Organophosphorate
	Metamidofos	Organophosphorate
	Cypermethrin	Pyrethroid
	Abamectin	Avermectins
	Trichlorfon	Organophosphorate
	Archer plus	Pyrethroid
	Actara	Neonicotinoids carbamate
	Furadan	Urea
Herbicides	Paraquat	Quaternary ammonium
	Diuron	Salt urea
	Glyphosate	Metribuzin
	Sencorex	Organophosphorate

The activity of AChE in human red blood cells (RBCs) may be considered as a biomarker for evaluating the central cholinergic status (Agrawal and Sharma 2010; Sharma et al. 2013; Gupta et al. 2015, 2016). As was already mentioned, pesticides can induce OS. Excessive production of reactive intermediates and ROS may cause DNA damage (Upadhyay et al. 2007, 2008), leading to a genotoxic effect. Our results showed a strong association between genotoxicity and neurotoxicity, as already reported (Muniz et al. 2008).

The susceptibility of RBCs and lymphocytes to OS due to pesticide exposure is a function of overall balance between the degree of OS and the antioxidant defense capability (Zeljezic et al. 2008; Prakasam et al. 2001). On the other hand, the low activity of RBCs observed not only indicates the intactness of RBCs but also acts as a viable biomarker to assess the extent of exposure of these cells to natural or anthropogenic chemicals (Callahan and Kruckenberg 1967). Inhibition of ACE activity in neurons as a result of exposure to the esters of phosphoric, sulfuric, and sulfonic acids and to carbamates represents a real parameter of toxicological stress. However, determination of such inhibition at neuronal level is practically impossible, but can be performed indirectly through the measurement of ACE activity in erythrocytes. This provides a direct measure of the damage caused, regardless of the type of ACE inhibitory chemical, and has proven to be the most appropriate indicator for the purposes of biological exposure monitoring (WHO 1986, 1996).

Insufficient protein synthesis in the hepatic parenchyma may decrease plasma or serum ACE activity by 30–40%. Thus, it is recommended to specifically determine ACE activity in erythrocytes in chronic exposure to cholinesterase inhibitors. In addition, alterations in AChE activity during OS have also been reported. The decrease in AChE activity might be related to the neuroimmunoregulatory role of this enzyme. Since both AChE and gamma glutamyl transpeptidase (GGT) are membrane-bound enzymes, GGT could interact with the amino acid neurotransmitter (acetylcholine) that may be removed from the binding with AChE and may result in AChE decreased activity (Koner et al. 1997).

Conclusion

Our results show a significant increase in CA and a significant decrease in AChE activity in farm workers. Thus, all the

people directly or indirectly involved in the handling of pesticides (including contractors and government officials) should be aware of the importance of using protective equipment and the potential hazards of occupational exposure to these carcinogenic agents. The regular training of occupational workers and the use of appropriate personal protective equipment should be recommended, together with the vigorous enforcement of strong pesticide safety regulations.

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Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflicts of interest.

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