Clinical, cytogenetic and toxicological studies in rural workers exposed to pesticides in Botucatu, São Paulo, Brazil

Estudos clínicos, citogenéticos e toxicológicos em trabalhadores rurais expostos a pesticidas em Botucatu, São Paulo, Brasil

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Abstract *Pesticides can cause gene mutations and chromosomal aberrations in exposed individuals. We have investigated 24 workers exposed to pesticides. Clinical examinations and cytogenetic and toxicological tests were performed. Ten non-exposed individuals were used as controls. Toxicological dosages of copper, zinc and manganese (metals found in some pesticides), hepatic enzyme dosage (GOT, GPT, AR) and acetylcholinesterase activity were performed in 16 workers and 8 controls. In the exposed workers, the most relevant clinical symptoms were poor digestion with fullness sensation after meals, irritated eyes, headache and fasciculations. The exposed group showed significantly lower manganese dosage and acetylcholinesterase activity, and significantly higher levels of alkaline phosphatase. Cytogenetic studies showed significantly higher chromosomal aberrations in the exposed group compared to the control group. Although the workers used protection against the pesticide's fog, the results revealed that the workers were contaminated with the pesticides. Therefore, the cytogenetic, toxicological studies with clinical examination are necessary for monitoring workers who are exposed to pesticides in any situation.*

Key words *Pesticides; Chromosome Aberrations; Toxicology; Contamination*

Resumo *Pesticidas podem causar mutações gênicas e aberrações cromossômicas em indivíduos expostos. Investigamos 24 trabalhadores expostos a pesticidas, nos quais foram executados exames clínicos e testes citogenéticos e toxicológicos. Dez indivíduos não expostos foram usados como controles. Dosagem toxicológica de cobre, zinco e manganês (metais encontrados em alguns pesticidas), dosagem de enzimas hepáticas (GOT, GPT, AP) e atividade de acetilcolinesterase foram executadas em 16 trabalhadores e oito controles. Nos trabalhadores expostos, os sintomas clínicos mais pertinentes foram digestão pobre, com sensação de plenitude após alimentação, olhos irritados, enxaqueca e fasciculações. O grupo exposto mostrou dosagem de manganês e atividade de acetilcolinesterase significativamente mais baixas, e nível significativamente mais alto de fosfatase alcalina. Estudos citogenéticos mostraram freqüências de aberrações cromossômicas significativamente mais altas no grupo exposto quando comparado ao grupo de controle. Embora usassem vestuário protetor contra névoa de pesticidas, o qual incluía calças de borracha, botas,luvas,máscara e chapéu, os resultados clínicos revelaram que os trabalhadores foram contaminados. Concluímos que estudos citogenéticos, toxicológicos, juntamente com exames clínicos, são importantes no controle da saúde do trabalhador, mesmo em condições de proteção.*

Palavras-chave *Praguicidas; Aberrações Cromossômicas; Toxicologia; Contaminação*

Introduction

Pathogen plagues and weeds cause low productivity and economic damage to the agriculture. Pesticides are an important way of control, but its extensive usage can cause damage to human beings exposed occupationally or by intake of contaminated water and food.

Pesticides include several categories of fungicides, insecticides, herbicides and others. Some fungicides include metals in its formulation, like copper, manganese, aluminum and zinc. Besides clinical effects, several metals have been described as dose dependent clastogenics and/or mutagenics: aluminium (Roy et al., 1990, 1991), copper (Flessel, 1977), stannic chloride (Ganguly, 1993), manganese (Joardar & Sharma, 1990), and zinc (Deknudt, 1982). Some pesticides showed no detectable effect. Organic pesticides can cause chromosome damage and several clinical symptoms to humans. There are many descriptions about the aneugenic, clastogenic, and sister-chromatid exchange effect of isolated pesticides.

Desi et al. (1990) described an increase in the frequency of hypodiploid cells in workers occupationally exposed to pesticides. De Ferrari et al. (1991) studying exposed individuals and bladder cancer patients who were exposed to pesticides, observed a significant increase in the frequencies of chromosomal aberrations and sister-chromatid exchanges in both groups. Surrallés et al. (1995) studying the induction of micronuclei of five pyrethroid insecticides in whole-blood and isolated human lymphocyte cultures, found clear dose dependent cytotoxic effects. They also found different genotoxic activity in vitro, depending of the pyrethroid insecticide used.

It is usual in the agricultural area to mix chemicals to treat the cultures against plagues. There is no description of cytogenetic, toxicological and clinical studies in humans exposed to some pesticides to observe possible synergistic effect, especially in individuals wearing protective garments.

In our project, the participants agreed to participate in the investigation by signing a document. The University Medical Ethical Council has approved the research project.

Material and methods

We have studied 34 male individuals, 24 being exposed and 10 controls, with mean age of 29.37 ± 9.01 and 39.00 ± 11.90 years old, respectively. The exposed group worked at a citrus Agricultural Company located at the Botucatu, State of Sao Paulo, Brazil. During the pesticides application, the workers used protective garments, rubber gloves, masks, and hats. The pesticides used were formicides, insecticides, fungicides and herbicides, alone or in mixture. Information on personal data, clinical signals, history of contact with pesticides, smoking and drinking habits, recent illnesses and treatments was obtained by a physician of our team during the clinical examinations. They also used an adequate protocol.

From each individual, 10 ml of peripheral blood was collected for the toxicological and cytogenetic studies.

Zinc and manganese dosages were made using an atomic spectrophotometer (GBC 932 AA with atomization by flame) and the dosage of the acetylcholinesterase activity in the plasma was made with a spectrophotometer 382 (Micronal) with 412nm wavelength. The dosages were performed at the Toxicological Assistance Center (CEATOX), Biosciences Institute, Paulista State University, Botucatu, São Paulo, Brazil.

The dosages of the hepatic enzymes glutamate oxalacetic transaminase (GOT), alkaline phosphatase (AP) and glutamate pyruvic transaminase (GPT) were made by a private laboratory.

The cultures for cytogenetic studies were made as described by the Moorhead's (1960) technique, with some modifications. Lymphocytes were cultivated at 37^{o}C in 3 ml of RPMI 1640 (Gibco) medium, supplemented with 10% of fetal calf serum and 0.1 ml of PHA-M (Gibco). The cells were harvested after 48 hours. Hypotonization was carried with 0.007 mM KCl and the fixation with methanol/acetic acid (3:1). The slides were air dried, stored at 4^{o}C and stained with Giemsa.

The slides were coded and examined in a blind test. Chromosomal aberration frequencies were determined for 24 exposed individuals, and the clinical examinations and the toxicological tests were performed for 16 of them.

The mitotic index (percent of cells in metaphase) was estimated in 100 cells/replicate. The frequencies of numerical and structural chromosome aberrations (break/gap, fragment, ring, centric fusion and rearrangement) were determined in 100 metaphases/individual.

Statistic evaluation was made using the "t" test for metals and enzyme dosages. The values for chromosome break, centromeric break and mitotic index were analysed by the Mann-Whitney U test, a non-parametric approach

indicated when the exact distribution of the variables is unknown (Carrano & Natarajan, 1988).

Results

The individual data about race, age, period of exposition, smoking and drinking habits, and clinical symptoms of the exposed and control groups are shown in Table 1. Several characteristics of both groups are shown in Table 2.

The individual cytogenetic results of both groups are in Table 3 and the mean and median data for aberrations are shown in Table 4. The exposed group showed an increased frequency of structural chromosome aberrations. Statistical analyses of the total break, centromeric breaks and Mitotic Index are shown in Table 5.

Table 1

Individual characteristics of the groups studied.

Individual*	Race**	Age (years)	Exposition (months)	Habits		Clinical symptoms
				Smoking	Drinking	
01E	B	38	120	+	+	Irritated eyes and gum, post prandial fullness sensation
02E	W	29	48	-	-	Sleepiness, irritated eyes, poor digestion
03E	W	32	84	+	+	None
04E	B	45	108	+	-	Irritated eyes, systemic arterial hypertension, headache, poor digestion, dizziness
05E	W	29	3	+	+	Stomach pain, irritated eyes
06E	W	54	78	-	-	Irritated eyes, systemic arterial hypertension, headache, fasciculation (arm), nasal bloody, tiredness, sleepiness
07E	B	34	54	+	+	Absence of appetite, fasciculation (face), tiredness
08E	W	29	36	-	-	Headache, irritated eyes, stomach pain
09E	B	31	48	-	+	Tiredness, irritated eyes
10E	B	18	25	-	-	None
11E	B	19	60	+	+	None
12E	W	22	16	+	-	Stomach pain
13E	W	18	48	+	+	Headache, stomach pain, fasciculation
14E	B	15	9	-	-	Dizziness, irritated eyes, headache
15E	W	29	48	+	+	Cramps, systemic arterial hypertension, poor digestion, irritated eyes
16E	W	19	24	+	-	Tiredness
17E	W	30	68	+	+	Fasciculation (arms), irritated eyes, abdominal pain (unspecific)
18E	B	24	72	+	+	Absence of appetite, irritated eyes, tiredness, fasciculation
19E	W	38	24	+	-	Poor digestion, heartburn
20E	W	29	12	-	-	None
21E	B	33	12	+	+	Absence of appetite, dizziness
22E	W	36	16	+	-	Absence of appetite, systemic arterial hypertension, headache, stomach pain
23E	B	26	24	-	+	Absence of appetite, sleepiness
24E	W	28	8	-	-	None
01C	W	25	0	+	+	None
02C	W	38	0	+	+	Stomach pain
03C	W	47	0	-	-	None
04C	W	40	0	+	+	None
05C	W	34	0	-	+	None
06C	B	60	0	-	-	None
07C	W	31	0	+	+	None
08C	W	32	0	-	+	None
09C	W	56	0	-	-	None
10C	W	27	0	-	-	None

* E = Exposed, C = Control.
** B = Black, W = White.

Table 2

General information about the groups.

	Control	Exposed
Number of individuals	10	24
Mean age	39.00 ± 11.90	29.37 ± 9.01
Smokers	4 (40%)	15 (62.5%)
Drinkers	6 (60%)	12 (50%)
Mean exposition in months	0	43.54 ± 32.20

Table 3

Individual distribution of structural and numerical chromosomal aberrations and mitotic index (MI). (E = Exposed, C = Control).

Individual	Structural aberrations							Aneuploidy			MI
	Gap	B'	B''	CB	Sub-total	Ring	Total	Hypo	Hyper	Total	
01E	02	–	03	17	20	–	22	01	02	03	08.11
02E	03	01	01	10	12	02	17	01	–	01	14.98
03E	–	–	–	04	04	–	04	–	01	01	13.99
04E	01	01	–	06	07	–	08	02	–	02	08.49
05E	–	–	01	06	07	–	07	01	–	01	17.54
06E	01	01	11	10	22	–	23	–	–	–	18.71
07E	–	–	–	04	04	–	04	–	–	–	05.91
08E	–	–	–	14	14	–	14	–	02	02	10.53
09E	–	–	03	03	06	–	06	–	–	–	04.82
10E	–	–	–	05	05	–	05	–	02	02	28.86
11E	–	–	–	01	01	–	01	–	–	–	16.91
12E	04	–	01	13	14	–	18	–	–	–	08.63
13E	–	–	03	16	19	01	20	–	01	01	11.63
14E	01	–	–	10	10	–	11	–	–	–	16.44
15E	–	–	07	05	12	–	12	01	–	01	05.61
16E	01	–	02	03	05	01	07	–	01	01	03.62
17E	–	–	–	07	07	–	07	–	–	–	12.66
18E	01	01	11	05	17	–	18	01	–	01	21.05
19E	–	–	01	01	02	–	02	–	–	–	12.29
20E	01	03	–	10	13	–	14	01	–	01	33.05
21E	01	–	03	18	21	–	22	–	–	–	07.60
22E	–	–	–	13	13	–	13	–	–	–	18.43
23E	–	01	01	19	21	–	21	–	–	–	01.77
24E	–	–	01	–	01	–	01	–	–	–	17.47
Total	16	08	49	200	257	04	277	08	09	17	
01C	02	01	–	02	03	–	05	–	–	–	06.52
02C	–	01	–	01	02	–	02	–	–	–	05.41
03C	–	–	–	–	00	–	00	–	–	–	06.51
04C	–	–	–	01	01	–	01	–	–	–	03.77
05C	–	–	01	–	01	–	01	–	01	01	05.66
06C	–	–	–	–	00	–	00	–	–	–	06.31
07C	–	–	01	02	03	–	03	–	–	–	03.38
08C	–	–	01	–	01	–	01	–	–	–	04.57
09C	–	–	–	–	00	–	00	–	–	–	03.46
10C	–	–	–	–	00	–	00	–	–	–	04.52
Total	02	02	03	06	11	00	13	–	01	01	

Table 4

Structural aberrations and mitotic index in the groups studied.

	Number of individuals	Number of metaphases	Total Breaks mean	Total Breaks median	Centromeric Breaks mean	Centromeric Breaks median	Mitotic Index mean	Mitotic Index median
Control	10	1,000	0.80 ± 1.03	0.5	0.60 ± 0.84	0	5.01 ± 1.24	4.99
Exposed	24	2,400	10.71 ± 6.77	11	8.58 ± 5.90	8	13.71 ± 7.23	12.47

Table 5

Statistical analysis of the total breaks, centromeric breaks, and mitotic index.

Variables	Z	Comparisons
Total breaks	4.23***	G1>G2
Centromeric breaks	4.15***	G1>G2
Mitotic Index	3.78***	G1>G2

G1 = exposed, G2 = control, *** p<0.001

Metal dosages and the determination of the acetylcholinesterase activity, along with the enzymes GOT, GPT, AP, ACHE, Cu++, Zn++ and Mn++ are shown in Table 6. Manganese dosage showed higher values in the control than in the exposed group. Statistical analyses of the enzymatic and metals dosage are in Table 7. Alkaline phosphatase dosage was significantly lower in the exposed group. Acetylcholinesterase activity is significantly higher in the control group ($t = 2.309$, $p<0.05$).

The clinical examination revealed difficulty for digestion represented by a fullness sensation after meals (57.5%), as well as irritated eyes (33.33%), headache (29.17%), tiredness, fasciculation and absence of appetite (20.83%), systemic arterial hypertension (25.00%) and an irregular contraction of the muscle fiber, mainly in the arms.

Discussion

Our study has demonstrated several clinical and toxicological problems, as well as an increase in the frequency of chromosome aberrations in workers exposed to pesticides in comparison with non-exposed people.

Several precautions were taken to protect the workers against pesticides fog with protective garments, gloves rubber boots, and masks. They were also instructed to always take a shower after each application of pesticides.

Besides all this, contamination has occurred, as revealed by the presence of several symptoms of chronic intoxication. The main symptoms observed were related to digestion and eye irritation. People had headaches, arterial hypertension, tiredness, fasciculation and absence of appetite.

According to Rolak (1993), fasciculation presented by the exposed group can be explained by a situation of hypomangnesemeses presented by them in comparison with the control group. In our study, the exposed group showed no significant difference of the dosage of copper and zinc compared to the control group. Unless the workers had an indication of hepatic alterations, it is difficult to say that the symptoms related to the digestion were caused by metal contamination.

The levels of two enzymes (acetylcholinesterase and alkaline phosphatase) were found to be altered in the exposed group. Acetylcholinesterase activity was low in both groups studied, but in the exposed group it was significantly lower than in the control group. The level of this enzyme is lower when there are hepatic lesions caused by contamination with organophosphate insecticides or cyclophosphamide, or in hepatic lesions with necrosis. Larini (1993) has described an inhibition of the sterase center of acetylcholinesterase by the insecticides. Its function is to avoid that the acetylcholine splits into choline and acetic acid. The lower acetylcholinesterase activity in the control group can be explained by the chronic ingestion of alcoholic beverages. This group was selected to be as much as possible similar to the exposed group, with the exclusion, of course, of the exposure to pesticides. Therefore, they also could have been influenced by that factor, revealed by the low level of the enzyme.

Low and moderate elevation of the alkaline phosphatase can occur in the serum of patients with hepatic dysfunction. In the exposed group, the level of this enzyme was significantly elevated in comparison to the control group.

Table 6

Individual data of the enzymatic dosage of GOT, GPT, AP, ACHE, Cu++, Zn++ and Mn++ according the smoking and drinking habits in both groups exposed and control.

Groups	Individual	GOT	GPT	AP	ACHE	µL/ml Cu++	Zn++	Mn++
Exposed								
S/D	01	32	30	93	1,5	14.8	295.0	4.4
	03	18	15	58	1,8	21.7	186.5	2.5
	05	26	43	76	1,5	18.5	292.9	2.1
	07	20	22	76	1,5	17.6	211.0	3.7
	11	20	15	146	0,7	17.6	210.5	2.6
	13	24	22	146	2,0	19.5	224.5	0.3
	15	18	60	146	1,4	16.6	258.0	3.5
NS/D	09	18	10	96	1.1	21.5	207.5	3.8
S/ND	04	16	20	136	1.7	21.3	307.2	2.3
	12	20	22	102	1.1	14.0	140.7	0.9
	16	20	20	76	1.8	9.6	124.3	3.9
NS/ND	02	22	105	120	1.1	16.6	197.0	1.0
	06	12	16	102	1.6	20.1	165.0	0.8
	08	16	15	120	1.4	13.1	171.0	2.8
	10	16	15	98	1.6	18.5	236.0	2.3
	14	08	20	63	1.5	14.7	174.7	0.8
Control								
S/D	01	10	12	44	2.0	13.4	198.2	4.7
	02	20	22	134	2.5	25.9	251.1	4.7
	04	14	15	72	1.4	15.9	124.9	4.3
	07	16	20	49	1.5	15.7	134.1	4.0
NS/D	05	10	18	29	1.9	11.3	213.0	3.7
	08	24	40	120	2.0	9.3	158.4	4.5
NS/ND	03	12	10	58	1.9	18.3	215.6	4.6
	06	10	15	40	13	9.1	197.7	4.5

S/D = smokers, drinkers; S/ND = smokers, non-drinkers; NS/D = non-smokers, drinkers; NS/ND = non-smokers, non-drinkers;
GOT = glutamic oxalacetic transaminase; GPT = glutamic piruvic transaminase; AP = alkaline phosphatase; ACHE = acetylcholinesterase activity.

Table 7

Statistical analysis of the enzymatic and metal dosage in the groups studied.

	Control	s	Exposed	s	t
GOT	14.4	(5.2)	19.1	(5.5)	1.965
GPT	19.0	(9.4)	28.1	(24.0)	1.326
AP	68.3	(38.6)	103.4	(29.7)	2.472*
ACHE	1.81	(039)	1.46	(0.33)	2.309*
Cu++	14.86	(5.53)	17.23	(3.37)	1.309
Zn++	186.63	(43.61)	218.91	(54.27)	1.458
MN++	4.37	(0.36)	2.36	(1.29)	5.793***

GOT = glutamic oxilacetic transaminase; GPT = glutamic piruvic transaminase;
AP = alkaline phosphatase; ACHE = acetylcholinesterase activity.

The transaminases level did not differ from one group to the other, although the level was higher in the exposed group. Elevated levels of those enzymes in serum indicate some type of hepatic alteration. In this case, there is no difference between the groups. Only the Mitotic Index was significantly elevated in the exposed group. This fact is not easy to explain. Ortiz (1995) discusses that malnourished children presented higher response to phytohemaglutinin stimulation than normal children. When those children were renourished, the stimulation index remained high, mainly after 72 hours. In the present case, the culture medium could be richer than the internal medium of the workers. Apparently, it was not possible to detect any level of malnutrition in the exposed group as compared to the control group.

Meisner et al. (1992) treated mice with the herbicides Alaclor and Atrazine, and a mixture of both. He has observed an additive effect 30 days after the treatment, but less in 90 days. The animals had an elevation of the mitotic index after 90 days when compared to the control group. However, the control was treated with cyclosphosphamide, that is an immunosupressor.

Human lymphocytes from young and older people were treated "in vitro" with aluminium sulphate (Roy et al. 1990). They have observed an elevation of the mitotic index in the young group.

In the present case, there is more stimulation of the cells in culture with phytohemaglutinin in the exposed group than in the control. This fact could be explained by the elimination of the abnormal cells.

The frequency of chromosome breaks (total and centromeric) was significantly different for the exposed group as described in the literature (De Ferrari et al., 1991).

According to this result, it is possible to conclude that it is necessary to have a follow up study in order to determine liver alterations. Although the workers have used protective garments, boots rubber, glasses and hats, many of them did not shave themselves every day and masks did not shut very well on their face. Contamination can occur through this groove.

As a recommendation, we could say that people working with pesticides should be monitored clinically, toxicologically and cytogenetically. This can assist any follow up studies showing possible contamination by pesticides. Of course, several other variables, such as smoking habits and alcoholic drinking can act in synergistic way.

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