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Genotoxic biomonitoring study of population residing in pesticide contaminated regions in Göksu Delta: Micronucleus, chromosomal aberrations and sister chromatid exchanges

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Abstract

Pesticides are widely used throughout the world in agriculture to protect crops and in public health to control diseases. Nevertheless, exposure to pesticides represents a potential risk to humans. This paper describes a study of possible genetic damage in the people living in regions contaminated with complex mixture of pesticides in Göksu Delta. In this study, used methods were chromosomal aberration (CA), sister chromatid exchange analysis (SCE) in the peripheral blood lymphocytes, and micronucleus (MN) assay in the buccal epithelial cells. In the present investigation, 32 affected subjects consist of 16 smoking and 16 non-smokings and an equal number of control subjects were assessed for genome damage. Micronucleus (MN), Broken egg (BE), Karyorrhexis (KR), Karyolysis (KL) and Binucleus (BN) frequencies were higher in affected subjects than in controls. Smoking had a statistically significant effect on the Micronucleus, Karyorrhexis and Binucleus frequencies for both the control and the exposed group. Also smoking and exposure affected the frequency of sister chromatid exchange and chromosomal aberrations compared with control groups.

Keywords: Genotoxicty; Micronucleus; Pesticide exposure; Smoking; Sister chromatid exchange

1. Introduction

A considerable number of synthetic organochlorine pesticides have been produced and offered for usage against pests since the 1940s. Especially after 1980, items primarily consumed by humans, including fish, mussels, and milk, have been analysed for organochlorine (OC) insecticide residues, and the results for the 1980s have been published or reported (Forget et al., 1993). According to 1997 market estimates, approximately 5684 million pounds of active pesticide ingredients are annually applied in the world (USEPA, 2001). The World Health Organization (WHO, 1992) reported that roughly three million pesticide poisonings occur annually and result in 220,000 deaths worldwide. Since 1945, more than 15,000 individual chemicals and 35,000 formulations have been used as agricultural pesticides (Forget et al., 1993). The chemicals are important tool for controlling agricultural pests, but they also represent a significant source of occupational and non-occupational exposure to potentially toxic agents. Research of other authors pointed out that some pesticides have genotoxic properties (Garrett et al., 1986; Sinha, 1989; Dunkelberg et al., 1994; Kevekordes et al., 1996; Gebel et al., 1997).

Turkey is a land of agriculture, so agricultural pest control is essential, and the most effective method for agricultural pest control is to use chemicals. Göksu Delta is an important wetland (13 000 ha) where the Göksu River reaches to sea in the eastern of town Tasucu-Ice1 (Ayas et al., 1997). There are two aquatic ecosystems in Göksu Delta, Paradeniz Lake and Akgöl Lagoon. The north of the lakes and eastern part of delta consist of farmland where rice, cotton and peanuts are grown all year.

In the previous studies, data showed that about 94 tons of pesticides and 431 tons of mineral fertilizers were used within 1 year at Göksu Delta (Çetinkaya, 1996). Ayas et al. (1997) reported that various environments and organisms were contaminated by 13 different pesticides and their residues. It was determined that the use of pesticide in Mediterranean region is more than average consumption of Turkey (Dalen et al., 2005).

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Table 1
Pesticides commonly used in Göksu Delta in 1999 (kg) (Erdoğan and Karaca, 2001)

Chemical group	Name	Active ingredient (kg)
Organochlorine	Endosulfane	1496
Organophosphates	Azynphos-methyl	137
	Chlorpyrifos-ethyl	4878
	Diazinon	268
	Dichlorvos	820
	Malathion	289
	Methamidophos	1698
	Methidathion	907
	Monocrotophos	254
	Parathion-methyl	3870
	Phoshamidon	340
	Trichlorfon	80
	Total	13541
Carbamates	Carbaryl	1029
	Carbosulfan	1136
	Primicarb	100
	Total	2265
Synthetic pyrethroids	Bifenthrin	38
	Cypermethrin	404
	Deltamethrin	641
	Lambda-cyhalothrin	485
	Lambda-cyhalothrin-buprofezin	71
	Total	1639
Benzoyl ureas	Chlorfluazuron	96
	Hexaflumuron	52
	Total	148
Others	Acetamiprid	86
	Chlorfenapyr	230
	Cyromazine	64
	Imidacloprid	471
	Thiocyclan hydrogen oxalate	323
	Pymetrozine	–
	Total	1174
Total		20263

Erdoğan and Karaca (2001) reported that this level is 9.9 kg per ha and 102 different types of pesticides were used in agriculture areas in Göksu Delta. They determined that the use of pesticide increased in rate of 11% in 1999 with respect to 1996. Also, in same article authors indicated that endosulfane pesticide was the used extensively pesticide among organochlorines in their report.

The exposure to pesticides has been associated with an increase in the incidence of various diseases such as non-Hodgkin's lymphoma (Zheng et al., 2001) pancreatic, stomach, liver, bladder and gall bladder cancer (Ji et al., 2001; Shukla et al., 2001). Toxicological evidence of mutagen and carcinogen action of several pesticides and the fact that large populations exposed to these compounds have attracted the attention of many cytogenetic studies (Sinha, 1989; Torres et al., 1992; Kevekordes et al., 1996; Gebel et al., 1997). A limited number of field studies seeking to evaluate the genetic risk of occupational exposure established an association between occupational exposure to complexes of pesticides and the presence of chromosomal aberrations (CAs) and/or sister chromatid exchanges (SCEs) and/or micronuclei (MN) (Kourakis et al., 1992; Carbonell et al., 1995; Ribas et al., 1996; Garaj-Vrhovac and Zeljezic, 1999). The role of MN as an intermediate endpoint of carcinogenesis has received much support in the literature, but stronger evidence is available concerning the association between rate of structural CA and cancer risk (Hagmar et. al., 2004). Genotoxic agents have the potential to interact with DNA and may cause damage to DNA. SCE occurs spontaneously in proliferating cells and is regarded as a manifestation of damage to the genome. It has been commonly used as a test of mutagenicity in order to evaluate cytogenetic responses to chemical exposure, and dose–response relationships for different chemicals have been reported in both *in vivo* and *in vitro* studies (Bal et al., 1998). In view of these findings, the detection of populations at risk constitutes a very important topic. In this context, it must be pointed out that cytogenetic markers such as CA, SCE and MN, have been extensively used for the detection of early biological effects of DNA-damaging agents. Human biological monitoring is a tool of great interest in cancer risk assessment and it allows estimating genetic risk resulting from environmental exposure to chemicals (Bonassi and Au, 2002).

At present, many biomarkers are used to determine the exposure levels to genotoxic agents, as well as their related effects. Micronucleus (MN) test has many advantages: reliable identification of cells that have completed only one nuclear division, sensitivity and precision, quickness and simplicity, the ability to screen large numbers of cells, and good reproducibility (Norppa et al., 1993; Fenech, 1997). MN, which appear in the cytoplasm of the divided cells as small additional nuclei, result from chromosome fragments or whole chromosomes that are left behind during mitotic division. Thus, the presence of MN is an indication of exposure to clastogenic and/or aneugenic agents. MN, in epithelial cells, offer many advantages in the biomonitoring of human populations exposed to suspected genotoxins (Burgaz et al., 1999; Karahalil et al., 1999; Pastor et al., 2003) although negative results have also been reported in different populations with occupational pesticide exposure (Davies et al., 1998; Lucero et al., 2000; Pastor et al., 2001). Similarly, MN in both buccal and urothelial epithelial cells have been used in the biomonitoring of agricultural workers exposed to pesticides (Pastor et al., 2002). Although only a few studies have been conducted, data reported using these cells also indicate both positive (Gomez-Arroyo et al., 2000) and negative results (Lucero et al., 2000; Pastor et al., 2001). Exfoliated buccal cells have been widely used in cytological evaluations to detect abnormal

Table 2
General characteristics of the affected and control groups

Study group	*N*	Age (years)	Years of exposed	Cigarette/day
		mean±SD	mean±SD	mean±SD
Control				
Smokers	16	34.31±6.32	–	16.31±4.94
Non-smokers	16	34.81±5.70	–	–
Total	32	34.56±5.92	–	16.31±4.94
Exposed				
Smokers	16	36.06±6.51	34.31±10.00	20.93±6.38
Non-smokers	16	36.37±7.49	34.81±11.24	–
Total	32	36.21±6.90	34.56±10.47	20.93±6.38

N, number of subjects; SD, standard deviation.

Table 3
The frequency of micronucleus, nuclear abnormalities and total nuclear changes in control groups

ID no.	The number of examined cell	MN (‰)	NCs				TNCs
			KL (‰)	KR (‰)	BN (‰)	BE (‰)	
Non-smokers							
1	3000	3	4	4	2	1	11
2	3000	2	3	4	3	1	11
3	3000	1	4	2	1	1	8
4	3000	1	4	2	2	1	9
5	3000	1	3	4	1	–	8
6	3000	5	2	4	2	1	9
7	3000	4	3	4	4	1	12
8	3000	4	4	4	3	–	11
9	3000	1	4	4	2	–	10
10	3000	5	3	3	2	1	9
11	3000	4	3	4	4	1	12
12	3000	1	3	4	1	–	8
13	3000	2	3	4	3	1	11
14	3000	5	3	3	2	1	9
15	3000	1	3	4	1	–	8
16	3000	5	3	3	2	1	9
Smokers							
1	3000	3	7	8	7	2	24
2	3000	3	8	8	8	3	27
3	3000	7	9	8	8	4	29
4	3000	3	5	8	8	8	29
5	3000	5	8	8	12	4	32
6	3000	4	9	9	15	6	39
7	3000	14	8	12	16	8	44
8	3000	11	10	10	16	7	43
9	3000	3	8	8	8	3	27
10	3000	3	8	6	12	8	34
11	3000	11	10	10	16	7	43
12	3000	11	10	10	16	7	43
13	3000	14	8	12	16	8	44
14	3000	3	8	8	8	3	27
15	3000	5	4	5	8	9	33
16	3000	3	8	8	8	3	27

ID: Idendification number; BE: Broken egg; BN: Binucleated; KL: Karyolysis; KR: Karyorrhexis; MN: Micronucleus; NCs: Nuclear changes; TNC: Total nuclear changes.

morphology, premalignant changes, and cancer. Since most cancers arise in epithelial tissue, exfoliated epithelial cells may be particularly useful for monitoring human populations exposed to various hazardous agents (Guzman et al., 2003; Cairns, 1975).

The inconsistent responses among studies could reflect different exposure conditions, such as exposure magnitude, the use of protective measures, and the specific genotoxic potential of the pesticides used, which indicate that data obtained from one population cannot be directly used for risk estimations in other affected groups. These factors make it necessary to study many well-defined populations in order to have a general view of the genetic risk associated with pesticide exposure.

The aim of study was to estimate the genotoxic risk in humans exposed to pesticide mixtures and to investigate the additive effect of smoking habit. This paper aims to present a global analysis of the populations included in the study, the goal of which was to determine if pesticide exposure is reflected in an increase in cytogenetic damage. Taking into account both the possible genetic risk of pesticide exposure and the advantages of using MN, CA and SCE as biomarkers, we report here the results obtained from biomonitoring a population from Turkey that is non-occupational exposed to pesticide.

2. Materials and methods

2.1. Subjects

In the beginning of the study, all the individuals signed an informed consent form and filled in a detailed questionnaire enquiring into information about possible confounding factors such as age, gender, smoking and drinking habits, vaccination, medication, X-ray examinations and diet. In the case of the affected group, years of exposure (living in pesticide contaminated region), protective measures used, *etc.*, were also recorded.

Table 4
The frequency of micronucleus, nuclear abnormalities and total nuclear changes in group affected from pesticide

ID no.	The number of examined cell	MN (‰)	NCs				TNCs
			KL (‰)	KR (‰)	BN (‰)	BE (‰)	
Non-smokers							
1	3000	4	7	8	10	6	31
2	3000	6	9	8	14	12	49
3	3000	4	6	6	10	10	36
4	3000	6	8	8	11	6	39
5	3000	4	7	7	9	9	36
6	3000	4	6	6	8	8	32
7	3000	5	8	8	9	10	40
8	3000	4	5	5	11	6	31
9	3000	4	6	7	10	11	38
10	3000	5	6	6	11	7	35
11	3000	7	8	8	10	7	40
12	3000	4	5	7	9	9	34
13	3000	5	8	7	11	6	37
14	3000	3	4	5	5	8	25
15	3000	6	7	7	8	8	36
16	3000	4	7	8	11	12	42
Smokers							
1	3000	8	6	9	12	10	
2	3000	6	9	11	13	13	52
3	3000	6	9	9	14	11	49
4	3000	6	9	10	12	11	48
5	3000	6	8	8	9	6	37
6	3000	8	9	12	13	6	48
7	3000	5	8	7	12	8	40
8	3000	6	7	8	10	9	40
9	3000	8	8	6	12	7	41
10	3000	8	10	9	14	8	39
11	3000	7	9	11	12	11	50
12	3000	7	8	10	12	13	50
13	3000	8	7	11	13	14	53
14	3000	6	9	9	13	12	49
15	3000	7	9	12	13	7	48
16	3000	5	5	7	12	7	36

ID: Idendification number; BE: Broken egg; BN: Binucleated; KL: Karyolysis; KR: Karyorrhexis; MN: Micronucleus; NCs: Nuclear changes TNC: Total nuclear changes.

Table 5
The frequencies of SCE and CA in the peripheral blood lymphocytes of group affected from pesticide

ID no.	The number of examined cell	SCE/M	MI (%)	RI (%)	TSCAs	TNCAs
Non-smokers						
1	100	9.02	4.16	1.93	6	2
2	100	8.26	4.06	1.97	8	4
3	100	8.22	3.50	1.89	7	3
4	100	9.02	3.21	1.92	5	2
5	100	8.44	4.22	1.97	5	2
6	100	7.50	4.86	1.97	4	1
7	100	7.20	6.12	1.99	4	2
8	100	9.10	4.92	1.98	3	2
9	100	8.30	4.86	1.95	4	2
10	100	6.69	6.02	2.06	5	3
11	100	8.56	5.10	1.86	5	2
12	100	8.40	4.20	2.00	5	2
13	100	8.12	4.00	1.84	4	2
14	100	7.12	5.61	1.97	5	2
15	100	8.19	4.17	1.93	5	3
16	100	8.62	4.11	1.92	4	2
Smokers						
1	100	9.16	4.22	1.85	2	2
2	100	8.21	3.12	1.84	8	11
3	100	7.11	4.00	1.89	10	5
4	100	6.51	3.25	1.86	6	2
5	100	8.21	4.11	1.92	9	3
6	100	8.61	5.02	1.85	7	3
7	100	8.00	4.00	1.95	8	2
8	100	9.02	3.00	1.92	4	2
9	100	8.61	4.25	1.80	5	2
10	100	8.41	4.20	1.79	3	3
11	100	7.52	4.00	1.85	5	3
12	100	8.82	4.00	1.82	4	3
13	100	6.61	4.00	1.83	4	2
14	100	8.30	3.50	1.89	3	2
15	100	9.14	3.00	1.89	4	2
16	100	9.01	3.00	1.85	4	3

ID: Idendification number; M: Metaphase; MI: Mitotic index; RI: Replication index; SCE: Sister chromatid exchange; TNCAs: Total numerical chromosome aberrations; TSCAs: Total structural chromosome aberrations.

To evaluate lifestyle factors, donors were stratified as smokers or non-smokers. Smokers were current smokers and smoked between 1 and 20 cigarettes/day. Only three from current smokers smoked more than 20 (22–25 cigarettes) cigarettes/day.

The study was carried out on 32 male affected subjects (16 smokers and 16 non-smokers) living in region contaminated with pesticide in Göksu Delta. All subjects were affected with a complex mixture of pesticides. In 1999, the extensively used pesticides are shown in Table 1. The control group also consisted of 32 healthy men (16 smokers and 16 non-smokers) who had no history of exposure to chemicals or other potentially genotoxic substances. In both control and exposed group, all subjects were men whose ages ranged from 25 to 45 years. The affected and controls subjects had not been taking medicine and had not been exposed to any kind of radiation (diagnostic and therapeutic) for 12 months before the blood sampling. The purpose of the study was carefully explained to the participants before they were asked to sign an informed consent form and to complete a standardized questionnaire to obtain data on lifestyle and personal factors (age, employment history, health, smoking habits, *etc.*). This study was conducted with the approval of our institutional human subjects committee. Smoking history recorded the use of cigarettes, cigarillos, cigars, and pipe tobacco over the lifetime. Neither of the study groups differed with regard to any lifestyle or personal factor. The demographic characteristics of the study groups are summarized in Table 2.

2.2. Cytogenetic method: sister chromatid exchange and chromosomal aberrations

Two tests were performed: chromosomal aberrations and sister chromatid exchange analysis. Blood samples were collected from affected subjects and controls by venipuncture. For each donor, two lymphocyte cultures were separately set up for both tests. Lymphocyte cultures were prepared according to the developed technique by Moorhead et al. (1960) with slight modifications. Heparinized whole blood (0.8 mL) was added to 5 mL of culture medium F10 (Gibco), supplemented to 18.5% with fetal calf serum (Gibco), with 0.2 mL phytohemagglutinin (Gibco), and with antibiotics (10,000 IU/mL penicillin and 10,000 IU/mL streptomycin). 5-Bromo-2-deoxyuridine (9 mg/mL; Sigma), used for SCE analysis, was added to cultures at the beginning of the 72-h incubation period at 37 °C. For both the SCE and, CA analysis, lymphocytes were cultured in the dark for 72 h and metaphases were blocked during the last 1.5 h with colcemid at final concentration of 0.2 mg/mL. The mitotic index was calculated to detect cytotoxicity as the proportion of metaphases among the total cell population by counting a total of 1000 cells. In addition to SCEs, cells were analyzed for the relative frequency of first division metaphases (M1; identifiable by uniform staining of both sister chromatids), second division metaphases (M2; identifiable by differential staining of the sister chromatids), and third and subsequent division metaphases (M3; identifiable by nonuniform pattern of

Table 6
The frequencies of SCE and CA in the peripheral blood lymphocytes of control groups

ID no.	The number of examined cell	SCE/M	MI (%)	RI (%)	TSCAs	TNCAs
Non-smokers						
1	100	4.02	5.05	2.10	–	–
2	100	4.88	5.02	2.05	3	–
3	100	3.96	6.02	2.02	–	–
4	100	5.06	5.98	2.11	–	2
5	100	5.10	5.45	2.05	2	–
6	100	5.92	5.48	2.02	1	–
7	100	4.86	5.47	2.05	1	–
8	100	6.02	5.23	2.05	–	–
9	100	5.94	4.86	2.03	1	–
10	100	5.96	5.32	2.04	1	–
11	100	6.06	5.46	1.99	1	2
12	100	5.04	5.68	2.00	1	–
13	100	4.80	6.01	2.00	1	–
14	100	3.08	6.10	2.01	1	–
15	100	4.10	5.00	2.06	1	–
16	100	4.62	5.13	2.00	1	–
Smokers						
1	100	5.96	4.11	1.98	2	1
2	100	5.12	4.23	1.98	4	2
3	100	7.06	4.52	1.99	2	–
4	100	5.08	4.47	2.00	3	–
5	100	4.94	4.58	2.00	3	–
6	100	5.98	4.96	2.01	2	–
7	100	5.05	5.01	2.00	1	1
8	100	6.08	4.36	1.98	2	–
9	100	6.92	4.66	1.98	1	–
10	100	7.90	5.01	1.99	2	–
11	100	6.96	5.12	2.01	1	1
12	100	4.12	4.98	2.00	2	1
13	100	6.16	4.66	2.01	1	1
14	100	4.02	5.41	1.97	2	–
15	100	6.19	5.44	1.95	1	–
16	100	6.12	4.53	1.99	2	1

ID: Idendification number; M: Metaphase; MI: Mitotic index; RI: Replication index; SCE: Sister chromatid exchange; TNCAs: Total numerical chromosome aberrations; TSCAs: Total structural chromosome aberrations.

Table 7
Frequencies (‰) of micronuclei and other nuclear changes in exfoliated buccal cells of control and affected subjects

Group	Smoking status	N	MN (‰)	KL (‰)	KR (‰)	BN (‰)	BE (‰)
Controls	Smokers	16	2.41±0.79	2.81±0.31	2.92±0.31	3.32±0.56	2.31±0.53
	Non-smokers	16	1.59±0.53	1.79±0.16	1.87±0.20	1.44±0.33	0.68±0.47
	Total	32	2.00±0.78	2.30±0.57	2.39±0.59	2.38±1.06	1.50±0.96
Affected	Smokers	16	2.57±0.20	2.84±0.24	3.03±0.30	3.49±0.19	3.06±0.43
	Non-smokers	16	2.15±0.24	2.57±0.26	2.62±0.20	3.11±0.32	2.88±0.35
	Total	32	2.36±0.31*	2.70±0.28*	2.83±0.32*	3.30±0.32*	2.97±0.40*

All values are given as mean±SD.
All values were presented as square root transformation values.
BE: Broken egg; BN: Binucleated cells; KL: Karyolysis; KR: Karyorhexis; MN: Micronuclei; *$p<0.01$ compared to control, for totals only.

staining). RI or proliferation index is the average number of replications completed by metaphase cells and is calculated as follows:

$$RI = 1(\%\text{ first division metaphases}) + 2(\%\text{ second division metaphases}) + 3(\%\text{ subsequent division metaphases})/100$$

Chromosomal aberrations were evaluated in 72-h whole blood cultures according to standard protocol (IAEA, 1986). The cells were collected by centrifugation, resuspended in a prewarmed hypotonic solution (KCl, 0.075 M) for 15 min, and fixed in methanol/acetic acid (3:1, v/v) solution (Carnoy's fixative). Air-dried preparations were made and the slides were stained by the fluorescence plus Giemsa procedure (Perry and Wolff, 1974) with slight modifications. Generally, for each donor, 100 cells at metaphase were analyzed for chromosome damage using cultures incubated for 72 h, and 100 well-spread metaphases containing 46 chromosomes in their second division that had clearly differentially stained were chosen to be counted for SCE frequency on coded slides. Cells including both numerical and structural chromosomal aberrations were recorded as aberrant cells. The slides were stained with 10% Giemsa dye solution. Chromatid breaks, chromosome breaks, fragments, dicentric, acentric chromosome and endomitosis and endoreduplications were evaluated as structural chromosomal aberrations and numerical chromosome aberrations, respectively.

2.3. Micronucleus test in buccal mucosa cells

Buccal epithelium cell samples were collected during the day. The subjects were asked to rinse their mouth with water before sampling. A wooden spatula was used to obtain buccal cell samples by gently scraping the roof of the mouth. The samples were then smeared onto clean microscope slides; three slides were prepared for each subject. The smears were air-dried and fixed in 3:1 (v/v) methanol:acetic acid. The slides were stained by the Feulgen reaction technique according to Stich and Rosin (1984), with slight modifications. Feulgen was preferred over Giemsa staining since Giemsa is less specific for DNA-containing structures. Three slides were prepared per subject. The authors evaluated 1000 cells per slide to determine MN, KR, KL, and BN frequencies. Nuclear changes (NCs) were classified according to Tolbert et al. (1992). Briefly, cells with two nuclei were considered as BN cells. Nuclei fragmented into irregular pieces were scored as KR. Nuclear dissolution, in which a Feulgen-negative, ghost-like figure of the nucleus remains, was evaluated as KL. Broken egg was nuclei that appeared cinched. For MN analysis, a MN must (1) be less than one-third the diameter of the main nucleus, (2) be on the same plane of focus as the nucleus, (3) have a chromatin structure similar to that of the main nucleus, (4) have a smooth, oval, or round shape, and (5) be clearly separated from the main nucleus.

2.4. Statistical analysis

All the data were analyzed using Generalized Linear Model. MN, KR, KL, BN, BE frequencies are expressed as the number per 1000 cells (‰), and given as means and standard deviations. A two-factorial experimental design was used to evaluate the data. Factor 1 was the group (level 1 = control, level 2 = workers) and Factor 2 was smoking status (level 1 = smokers, level 2 = non-smokers). Each combination included 16 subjects. Statistical analysis was performed using SPSS 12.0 for Windows (SPSS, Chicago, IL). $p<0.05$ was considered as the level of significance. Square root transformation was applied to the MN, BN, KR, KL BE, TSCA and TNCA frequency data before performing analysis of variance. Multivariate analysis of variance (MANOVA) was used to evaluate the MN, BN, KR, KL, BE, TSCA TNCA, SCE, MI, and RI data. The type III sum of squares method was used because it is a test of effects after controlling for all other factors and, in addition, it is easily interpreted. 95% confidence interval was used in the study.

3. Results

Table 3 represents the frequency of micronucleus, nuclear abnormalities consisting BE, KR, KL, BN, and total nuclear changes in control groups. Table 4 shows the frequency of micronucleus, nuclear abnormalities and total nuclear changes in group affected form pesticide. The frequencies of SCE and CA in the peripheral blood lymphocytes of group affected from pesticide and control group are described in Tables 5 and 6, respectively.

Table 7 represents the transformed values of micronuclei and other nuclear changes in exfoliated buccal cells of control and affected

Table 8
The values of cell kinetic and frequencies of SCE and TSCA, TNCA in peripheral blood lymphocyte cells of control and affected subjects

Group	Smoking status	N	MI (%)	RI (%)	SCE/M	TNCAs	TSCAs
Controls	Smokers	16	4.75±0.39	1.99±0.01	5.85±1.07	0.46±0.55	1.36±0.30
	Non-smokers	16	5.45±0.40	2.03±0.03	4.96±0.87	0.17±0.48	0.82±0.52
	Total	32	5.10±0.53	2.01±0.03	5.40±1.06	0.32±0.53	1.09±0.50
Affected	Smokers	16	3.79±0.58	1.86±0.04	8.20±0.85	1.70±0.48	2.26±0.50
	Non-smokers	16	4.57±0.83	1.94±0.04	8.17±0.70	1.48±0.22	2.20±0.26
	Total	32	4.18±0.81*	1.90±0.06*	8.18±0.77*	1.59±0.38*	2.23±0.39*

All values are given as mean±SD.
TSCAs and TNCAs were presented as square root transformation values.
M: Metaphase; MI: Mitotic index; RI: Replicative index; SCE: Sister chromatid exchange; TNCA: Total numerical chromosome aberrations; TSCA: Total structural chromosome aberrations; *$p<0.01$ compared to control, for totals only.

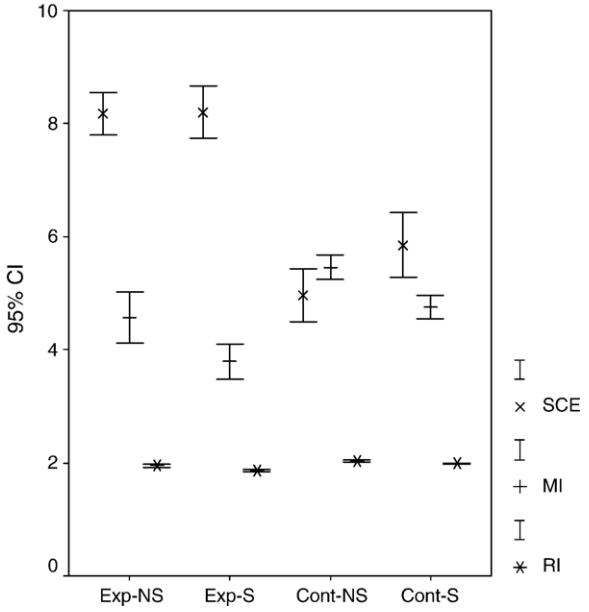

Fig. 1. Error bars representing the frequency of SCE, RI, and MI in peripheral blood lymphocytes of control and affected subjects. 95% CI: 95% Confidence interval.

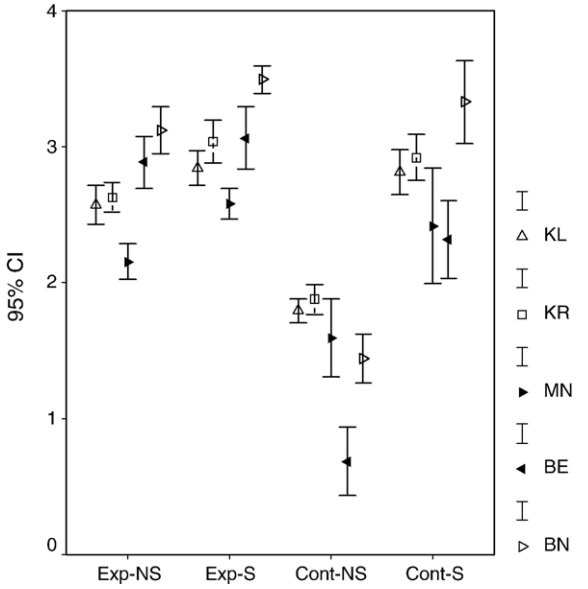

Fig. 3. Error bars representing the frequency of KL, KR, MN, BE, and BN in buccal epithelial cells of control and affected subjects. 95% CI: 95% Confidence interval.

subjects. The transformed values of cell kinetic and frequencies of SCE and TSCA, TNCA in peripheral blood lymphocyte cells of control and affected subjects are shown in Table 8.

MN, BE, KR, KL, BN, TSCA, TNCA, SCE and CA frequencies were higher in affected individuals than in non-controls (Figs. 1–3).

Regarding MN, KR, KL, BN no differences were found between the affected-smokers and control-smokers and no differences were found between the affected-non-smokers and control-non-smokers for BE frequency (Table 9 and Fig. 3). There is a significant difference between control-smokers and control-non-smokers for all parameters ($p<0.05$) (Table 9, Figs. 1, 2 and 3).

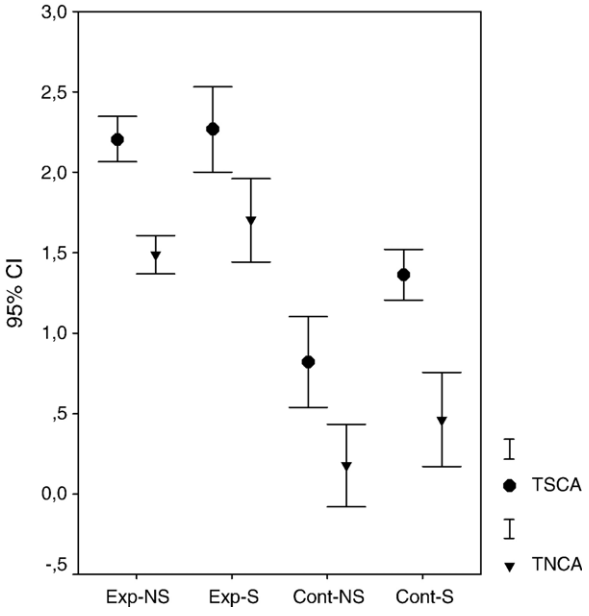

Fig. 2. Error bars representing the frequency TNCA of TSCA in peripheral blood lymphocytes of control and affected subjects. 95% CI: 95% Confidence interval.

4. Discussion

Pesticides constitute a heterogeneous category of chemicals, specifically, designed for the control of pests, weeds or plant diseases. Their application is still the most effective and accepted means for the protection of plants from pests, and has contributed significantly to enhanced agricultural productivity and crop yields. Many of these compounds, because of their environmental persistence, will linger for many years in our environment (Bolognesi, 2003). Many of these chemicals are mutagenic (Galloway et al., 1987; Garaj-Vrhovac and Zeljezic, 2000), linked to the development of cancers (Leiss and Savitz, 1995) or may lead to developmental deficits (Arbuckel and Server, 1998). Since several studies have shown that exposure to pesticides may induce genotoxic effects in occupationally

Table 9
Results of pairwise comparison of differences among groups and within groups

Groups	MN	BE	BN	KL	KR	MI	RI	SCE	TNCA	TSCA
Control-NS *vs* Affected-NS	*	n.s.	*	*	*	*	*	*	*	*
Control-S *vs* Affected-S	n.s.	*	n.s.	n.s.	n.s.	*	*	*	*	*
Control-S *vs* Control-NS	*	*	*	*	*	*	*	*	*	*
Affected-S *vs* Affected-NS	*	n.s.	*	n.s.	*	*	*	n.s	*	n.s

Affected-NS: Affected non-smoker; Affected-S: Affected-smoker; Control-NS: Control non-smoker; Control-S: Control-smoker; n.s.: non-significant; LSD values for BE, 0.264; BN, 0.219; KL, 0.147; KR, 0.153; MI, 0.339; MN, 0.294; RI, 0.026; SCE, 0.516; TNCA,0.263. TSCA, 0.242; *$p<0.05$.

exposed human populations (Börzsönyi et al., 1984; Dulot et al., 1985; Nehéz et al., 1988), the evaluation of the genotoxicity of pesticides in use is of immediate concern.

Smoking is another important variable for inducing significant alterations to the genetic material, as indicated by the increased frequency of SCE (Çelik and Akbaş, 2005), and, hence, increasing the risk of cancer. It has also been suggested that smoking increases the rate of MN formation in buccal epithelial cells (Çelik et al., 2003). In order to find whether there is a long-term effect of smoking in populations exposed to pesticides, we included non-smoker and current smoker groups in our study. Smoking also affected the genotoxicity and toxicity endpoints in both the exposed and control groups. Cigarette smoking is a known confounding factor that may influence the frequency of cytogenetic damage, such as chromosomal aberration, SCE, and MN formation in humans (Lakhanisky et al., 1993; Pitarque et al., 1997; Çelik and Akbaş, 2005). Smoking also was reported to increase the MN frequency in buccal cells (Piyathilake et al., 1995; Çelik et al., 2003). Our findings indicate that cigarette smoking significantly increases the frequencies of MN, KR, KL, BE, BN, TSCA, MI, and RI in both controls and exposed subjects. The living in areas polluted with pesticide has concealed the effects of smoking for SCE and TNCA. In the study performed by Pastor et al., the frequency of micronuclei (MN) in peripheral blood lymphocytes and in buccal epithelial cells was used as a biomarker of genotoxic effects resulting from occupational exposure to pesticides. In addition, the cytokinesis-block proliferation index (CBPI) was calculated to detect possible variations in the proliferative kinetics of lymphocytes due to pesticide exposure. This study was performed on 84 pesticide-exposed workers and 65 unexposed controls from Hungary. The pesticide-exposed workers, classified as moderately and highly exposed, were also evaluated separately. Smoking affected the frequency of MN in buccal cells. Results from the CBPI analysis showed that the proliferation index decreased with pesticide exposure and that this parameter was also affected by smoking and by the gender of individuals. The reduced CBPI in the highly exposed population suggests a possible genotoxicity of pesticide exposure.

There are conflicting reports in the literature about the effect of pesticide exposure on human populations, which may reflect the exposure conditions of the particular study population. Many researchers found positive associations between exposure to pesticides and cytogenetic damage (Gómez-Arroyo et al., 2000; Márquez et al., 2005). Márquez et al. (2005) found a significant increase in the frequency of binucleated cells with micronuclei (BNMN) was found in the exposed women as compared with the controls (36.94±14.47 *vs* 9.93±6.17 BNMN/1000 BN cells). In study performed by Gómez-Arroyo et al. SCE were analyzed in the peripheral blood of 30 persons, 22 women and 8 men, with 10 and 1.5 years of exposure to pesticides, respectively, and of 30 persons, 28 women and 2 men, that were considered as the non-exposed group. Samples of buccal mucosa were also taken from each person. Significant differences between exposed and non-exposed groups were found for SCE, RI and MI. Besides, the MN frequencies in the exposed group were three times higher than in the non-exposed group. Because micronuclei could be the consequence of the mitotic spindle malfunction it is possible that the mixture of pesticides could also express an aneugenic mode of action.

In the present study, the increase in the mean value of SCE, TNCA and TSCA in the affected group was significantly higher than in controls ($p<0.01$). In the affected group, it is found that SCE values were 8.18±0.77 while control group was 5.40±1.06. In the affected group, it is found that TNCA and TSCA values were 1.59±0.38 and 2.23±0.39 while control group was 0.32±0.53 and 1.09±0.50, respectively. MI and RI values decreased significantly in affected group compared with control group ($p<0.01$). While MI and RI values are 5.10±0.53 and 2.01±0.03 in control group, in affected group these values reached the 4.18±0.81 and 1.90±0.06 respectively. In this same way, MN, BE, KR, KL, BN frequencies significantly increased in affected group in comparison with frequencies in control groups ($p<0.01$). The MN, BE, KR, KL, BN frequencies 2.00±0.78, 1.50±0.96, 2.39±0.59, 2.30±0.57, 2.38±1.06 in control group and these values reached the 2.36±0.31, 2.97±0.40, 2.83±0.32, 2.70±0.28, 3.30±0.32 in affected group, respectively. Smoking and exposure one by one had a significant effect on the MN, KR, KL, BE, BN, TSCA frequencies, MI, and RI values. There is no interaction between cigarette and exposure for MN, KL, KR, and BN and it is worth noting that there is an interaction between cigarette and exposure for BE, TSCA, TNCA, SCE, MI, and RI. Totally, compared with control, the increases in all the parameters is statistically significant (Tables 7 and 8). In contrast to the affected group, cigarette smoking caused a statistically significant increase of SCE frequency and TSCA in control group (Table 9, Figs. 1 and 2). Similar results were obtained by other authors who showed that an increased number of SCE was found in subjects exposed to pesticides. Costa et al. evaluated cytogenetic damage in peripheral lymphocytes from 33 farmers of Oporto district (Portugal) exposed to pesticides by means of MN, SCE and found that MN and SCE frequencies were significantly higher in the exposed group. They determined SCE frequency and MN frequency are 5.19±0.20 and 4.33±0.19, respectively. Shaham et al. (2001) investigated the SCE in peripheral lymphocytes of 104 greenhouse farmers exposed to pesticides and 44 unexposed workers. They found that the evaluation of the influence of years of exposure on the frequency of SCEs showed that the frequency of SCEs were higher among those farmers who were exposed to pesticides for more than 21 years than among those with less than 21 years of exposure. The variables that had the most influence on the elevation of SCEs were self-preparation of the pesticide mixtures and the number of sprayings per year. Because the farmers used a mixture of almost 24 different chemical classes it was impossible to attribute exposure to a specific pesticide or group of pesticides to single farmers. Zeljezic and Garaj-Vrhovac (2002) performed a study related with subjects exposed to pesticide mixture. In exposed group, it was found that SCE frequency (5.79±0.81) was higher than control group (2.96±0.38). Considering the fact that genomic lesions that could lead to SCE are not lethal, lymphocytes containing this damage could remain in the blood

stream for several years. This fact is in agreement with the number of cells classified as high frequency cells (Carrano and Moore, 1982; Ponzanelli et al., 1997; Bonassi and Au, 2002).

Risk assessment of complex environmental samples suffers from difficulty in identifying toxic components, inadequacy of available toxicity data, and a paucity of knowledge about the behavior of geno(toxic) substances in complex mixtures. Lack of information about the behavior of toxic substances in complex mixtures is often avoided by assuming that the toxicity of a mixture is simply the sum of the expected effects from each mixture component, *i.e.* no synergistic or antagonistic interactions.

A major problem in interpreting biomonitoring studies is estimating the degree of exposure. Until now, many biomonitoring studies have been performed in people from different regions and under a variety of exposure conditions. In this context, it is not surprising that the results obtained by different authors have also shown a high variability. A major explanation for this variability may well lie in different assays employed and the levels of exposure. The variation in the degree of exposure and the use of different chemical mixtures may be a reason for conflicting results between studies.

In conclusion, this study showed an increased rate of MN, SCE and CAs in pesticide-exposed individuals and the finding of a significant increase of SCEs, MN and CAs frequency in peripheral lymphocytes of affected subjects refers to a potential cytogenetic hazard due to pesticides exposure. Particularly, also from previous studies, it is clear that the period of pesticide exposure is effective. In our study, cigarette smoking caused a significant effect on all investigated parameters ($p<0.05$). Our study represents an important contribution to the correct evaluation of the potential health risk associated with agrochemical exposure. In affected group, smoking has no significant effects on the BE, KL, SCE, and TSCA ($p>0.05$).

We all learn about risks at an early age—how to recognize them and how to avoid them. Some risks are obvious and immediate: proximity to hot stoves, use of chain saws, and driving on the highway. But other risks (especially those associated with cancer), like tobacco use, and pesticide and radiation exposure, result in delayed effects that are often hard to recognize. Understanding the influence of chemicals on genotoxic response is expected to improve the applicability of genotoxicity assays in biomonitoring and testing.

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