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Abstract

This study included 45 patients with intentional insecticide intoxication and 21 with accidental intoxication who were treated at the First-Aid and Emergency Department of Balcali Hospital at the Faculty of Medicine in the Cukurova University, Adana, Turkey, while the control group consisted of 25 people selected from university personnel known to be healthy. Patients with a history of X-ray exposure in the last 6 months or of any virus disease as well as continuous drug users and smokers were excluded, leaving a total of 49 patients. Acetylcholine esterase (Pseudocholinesterase) enzyme (AchE), sister-chromatid exchanges (SCE), the mitotic index (MI), and the replication index (RI) were evaluated. Blood samples were cultured for SCE evaluation and sera separated for AchE levels. Insecticide exposure was generally intentional for suicide in adolescents and at older ages, but accidental for children. AchE levels were found to be significantly lower in organophosphorus (OP) and carbamated (CB) insecticide poisoning groups in comparison with the control group ($p<0.001$), while the pyrethroid (PY) group was not statistically different for the AchE effect ($p>0.05$). SCE was found to be significantly higher in OP and CB groups ($p<0.001$), while the PY and control groups were statistically similar for SCE levels ($p>0.05$). This study showed an increase in SCE in response to orally ingested insecticides. These findings indicate that insecticide exposure results in cell abnormalities, with resulting impediments to the division and replication of cells, as suggested by MI decreases and RI increases, while the speed of the division cycles of stimulated cells increases.

KEYWORDS: insecticide intoxication, acetylcholine esterase enzyme (AchE), sister-chromatid exchanges (SCE)

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Original Article

The Sister-Chromatid Exchange and Acetylcholine Esterase Enzyme Levels among Patients with Insecticide Intoxication in the Çukurova Region, Turkey

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This study included 45 patients with intentional insecticide intoxication and 21 with accidental intoxication who were treated at the First-Aid and Emergency Department of Balcali Hospital at the Faculty of Medicine in the Çukurova University, Adana, Turkey, while the control group consisted of 25 people selected from university personnel known to be healthy. Patients with a history of X-ray exposure in the last 6 months or of any virus disease as well as continuous drug users and smokers were excluded, leaving a total of 49 patients. Acetylcholine esterase (Pseudocholinesterase) enzyme (AchE), sister-chromatid exchanges (SCE), the mitotic index (MI), and the replication index (RI) were evaluated. Blood samples were cultured for SCE evaluation and sera separated for AchE levels. Insecticide exposure was generally intentional for suicide in adolescents and at older ages, but accidental for children. AchE levels were found to be significantly lower in organophosphorus (OP) and carbamated (CB) insecticide poisoning groups in comparison with the control group ($p<0.001$), while the pyrethroid (PY) group was not statistically different for the AchE effect ($p>0.05$). SCE was found to be significantly higher in OP and CB groups ($p<0.001$), while the PY and control groups were statistically similar for SCE levels ($p>0.05$). This study showed an increase in SCE in response to orally ingested insecticides. These findings indicate that insecticide exposure results in cell abnormalities, with resulting impediments to the division and replication of cells, as suggested by MI decreases and RI increases, while the speed of the division cycles of stimulated cells increases.

Key words: insecticide intoxication, acetylcholine esterase enzyme (AchE), sister-chromatid exchanges (SCE)

The Çukurova Region, located in the south eastern region of Turkey along the Mediterranean Coast, is a place with widespread agricultural fields with a resulting high frequency of insecticide use and the presence of such chemicals at many houses. The insecticides used in the Çukurova Region cause intoxication by an oral route during the spraying of fields, or by respiratory or dermal routes. Improperly preserved insecticides inside houses are another source of intoxication either by accidental drinking, as in the case of children, or by intentional drinking for suicidal purposes.

The World Health Organization has declared insecticide intoxication to be an important public health problem, affecting nearly 3 million people in the world every year, resulting in 200,000 deaths. A large portion of these deaths is confined to third world countries, which use nearly 25% of all insecti-

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cides world wide [1–3].

This intoxication primarily occurs due to ignoring the directions for use, unsafe transport or storage, lack of protective clothing, including gloves or masks, during spraying, and insufficient first-aid or medical aid [3–6].

The insecticides, especially organophosphorus (OP) and carbamated (CB) insecticides, received by respiratory, enteric, or dermal routes block the nervous system by the inhibition of acetylcholine esterase enzyme (AchE), resulting in unconsciousness and death [4–8]. Insecticides are integrated into the cell structure, causing chromosomal abnormalities during cell division, sister-chromatid-exchanges (SCE), and nucleolus loss [9–14]. The cytogenetic effects of many OP, CP, and pyrethroid (PY) insecticides on some mammals other than human beings have been reported based on other studies [15–17].

Many insecticides affect both agriculture and public health in the Çukurova Region. The research performed to date remains insufficient, despite the known toxic effects of these insecticides on mammals and human beings [7,18,19].

In the present study AchE levels and chromosomal SCEs were examined in blood samples of patients accidentally or intentionally exposed to insecticides in agricultural fields or in houses and who applied to the First-Aid and Emergency Department of Balcali Hospital at the Faculty of Medicine in the Çukurova University.

Materials and Methods

Blood sampling and donor selection. The study group was divided in 2 groups: (1) The patient group consisted of 66 patients exposed to insecticides who applied to the First-Aid and Emergency Department of Balcali Hospital at the Faculty of Medicine of Çukurova University (Adana, Turkey) with acute symptoms of intoxication, like nausea, vomiting, parasympathetic signs, unconsciousness, and (2) the control group consisted of people selected from university personnel known to be healthy. The questionnaire form information and the verbal informed consent to participate in the study were obtained from participants or from accompanying persons in the case of unconsciousness. The age of the patient, means of intoxication, disease history in the last 6 months, information regarding continuously used drugs, exposure history to x-rays or any mutagenic chemicals in the last 6 months, and the history of smoking were investigated. Participants with a history of exposure to x-rays or to mutagenic chemicals or of any viral disease in the last 6 months as well as smokers and continuous drug users were excluded. Only 49 patients underwent AchE, SCE, mitotic index (MI), and replication index (RI) examination.

While none of the patients were regular users of insecticides (*i.e.* they were not insecticide sprayers or vendors), they can be assumed to have been chronically exposed to the insecticides, as they live on or nearby agricultural fields in the Çukurova Region (Turkey), where farming activities are performed throughout the entire year. The fields are sprayed by these chemical agents at different times of the year, with a resulting continuous presence of insecticides in domestic environments, as has been personally observed during various field surveys and screening activities performed by the Public Health Department for many years.

The participants were divided into 2 groups: the intoxication by accident group (Accidental Exposure = AE) and the intoxication by intention group (Intentional Exposure = IE). The age groups were a child-age group (0 to 14 years), an adolescent-age group (15 to 22 years), and an adult-age group (23 years and older).

Cell culture and slide preparation. Blood samples were collected within 1–3 h of exposure to insecticide, sent to the laboratory, and cultured in 1–2 h time according to the modified method of Perry and Wolff [20]. After excluding the first 3 drops in order to prevent any contamination, 6 drops of whole blood samples were collected in 10 ml gas-tight sterile glass culture tubes, containing 2.5 ml of Chromosome Medium B (Seromed Cat. No.: F-5023) and 50 μl of BrdU (5-bromo-2'-deoxyuridine, Sigma, Taufkirchen, Germany), prepared at a concentration of 10 μg/ml. The tubes were mixed in the palm, covered with aluminum foil for light-prevention, and incubated at 37℃ in a semi-inclined position. Then to each tube was added a total of 30 μl colcemide of 0.2 μg/ml concentration. After further 2 h incubation, hypotonic KCl (0.075M) solution vas applied to distend the leukocytes and to burst the erythrocytes.

The cells were fixed 3 times in methanol/acetic acid (3:1). Previously cleaned and chilled slides were used to examine 3–4 drops of leukocyte pellets.

Staining method. Slides stored in the dark were placed in a regular horizontal container covered with a thin film of soransen buffer and irradiated for 1 h under a UV lamp at a distance of 25 cm. The irradiated slides were placed for 1 h in 2xSSC buffer stored in a hot water basin at 60℃. Finally, the slides were rinsed with tap water and stained for 15 min by the Giemsa method (5%) [20].

Sister-chromatid exchange, replication index, and mitotic index. A minimum of 25 metaphase II (M_2) stages were examined for each patient, and SCE counts were recorded to determine the mean SCE count per metaphase plaque. RI was determined by examining 100 metaphase plaques, and the cells entering metaphase I (M_1), M_2 and metaphase III (M_3) were counted. The following formula was used to calculate the RI:

$RI = (M_1 \times 1 + M_2 \times 2 + M_3 \times 3) / 100$

MI was the percentage of mitotic cells among 1000 lymphocytes.

Determination of Acetylcholine Esterase Enzyme. The serum levels of AchE were determined by the routine method —S-butyrylthiocholine iodide method— of the Central Laboratory of the Hospital, working under continuous quality controls at ISO norms [21].

Statistical methods. The statistical methods used were the chi-square test, the Student t-test, multivariate analysis of variance with Tukey's HSD, and Tamhane post-hoc comparisons.

Results

Most of the patients intentionally or accidentally exposed to insecticides belonged to the adult age group (43.94%), followed by the adolescence (36.36 %) and child (19.69%) age groups (Table 1). When the exposure groups were separately examined, the intentional exposure group consisted predominantly of adults (51.11%), while the accidentally exposed group consisted predominantly of children (52.38%) (X_2=20.9, df=2, p<0.001).

The individual insecticide groups and the entire group were compared to the control group and among themselves AchE, SCE, MI, and RI means (Table 2).

The AchE mean of the insecticide group (4.37±1.65) was significantly lower than that of the control group (7.32±1.32) (p<0.05). The AchE means of the OP (3.87±1.04) and CB (3.98±1.07) groups were similar (p=0.99), but both were lower than the mean of the control group (p=0.001) and of the PY group (7.21±0.52) (p=0.001). The mean AchE of the PY

Table 1 The distribution of insecticide intoxication according to age group and reasons

Exposed to insecticides	0–14 age(Child) n(%)	15–22 age (Adolescence) n(%)	23 and above age (Adult age group) n(%)	Total n(%)
Intentionally	2 (4.44)	20 (44.44)	23 (51.11)	45 (100)
Accidentally	11 (52.38)	4 (19.04)	6 (28.57)	21 (100)
Total	13 (19.69)	24 (36.36)	29 (43.94)	66 (100)

Table 2 The distribution of the AchE, SCE, MI and RI values in the OP, CB, PY and control groups

	Organophosphate n=30		Carbamate n=12		Pyrethroid n=7		All Insecticides n=49		T test	Control n=25	
	X	SD	X	SD	X	SD	X	SD		X	SD
AchE	3.87	±1.04	3.98	±1.07	7.21	±0.52	4.37	±1.65	p<0.05	7.32	±1.32
SCE	7.51	±1.11	7.55	±0.90	5.81	±0.74	7.28	±1.17	p<0.05	5.52	±0.83
MI	3.67	±0.98	3.56	±0.57	5.17	±0.95	3.85	±0.15	p<0.05	5.60	±0.94
RI	2.59	±0.57	2.43	±0.26	2.32	±0.39	2.51	±0.63	p<0.05	2.26	±0.34

n, number of samples; X, Mean; SD, Standard Deviation.

group was similar to the mean of the control group (p=0.996) (Table 2, 3).

The SCE mean was significantly higher in the insecticide group (7.28±1.17) than in the control group (5.52±0.83) (p<0.05). The SCE means in the OP (7.51±1.11) and CB (7.55±0.90) groups were similar (p=0.99), but both were higher than those of the control group (p=0.001) and of the PY group (5.81±0.74) (for OP vs. PY; p=0.001 with Tukey's and p=0.002 with Tamhane) (for CB vs. PY; p=0.002 with Tukey's and p=0.003 with Tamhane). The SCE mean of the PY group was similar to that of the control group (p=0.898 with Tukey's and p=0.898 with Tamhane) (Table 2, 3).

The MI mean was significantly lower in the insecticide group (3.85±0.15) than in the control group (5.60±0.94) (p<0.05). The MI means of the OP (3.67±0.98) and CB (3.56±0.57) groups were similar (p=0.986 with Tukey's and p=0.99 with Tamhane), but both were lower than those of the control group (p=0.001) and of the PY group (5.17±0.95) (for OP vs. PY; p=0.001 with Tukey's and p=0.027 with Tamhane) (for CB vs. PY; p=0.002 with Tukey's and p=0.019 with Tamhane). The MI mean of the PY group was similar to the mean of the control group (p=0.698 with Tukey's and p=0.902 with Tamhane) (Table 2, 3).

The RI mean of the insecticide group (2.38±0.63) was significantly higher than that of the control group (2.26±0.34) (p<0.05). The RI means of the OP (2.59±0.57) and CB (2.43±0.26) groups were similar (p=0.732 with Tukey's and p=0.793 with Tamhane), but both were higher than those of the control group (p<0.05) and of the PY group (2.32±0.39) (p=0.001). The RI mean of the PY group was similar to that of the control group (p=0.001) (Table 2, 3).

Table 3 The comparison distribution of AchE, SCE, MI and RI means in the individual insecticide groups (OP, CB, PY) and the control group

				AchE	SCE	MI	RI
ONE WAY ANOVA			Means	55.894	24.553	25.525	13.131
			Significance	0.001	0.001	0.001	0.001
MULTIPLE COMPARISON TEST	TUKEY	1=OP	2	0.99	0.99	0.986	0.732
			3	*0.001	*0.001	*0.001	*0.001
			4	*0.001	*0.001	*0.001	*0.049
		2=CB	1	0.99	0.99	0.986	0.732
			3	*0.001	*0.002	*0.002	*0.001
			4	*0.001	*0.001	*0.001	*0.032
		3=PY	1	*0.001	*0.001	*0.001	*0.001
			2	*0.001	*0.002	*0.002	*0.001
			4	0.996	0.898	0.698	0.061
		4=Control	1	*0.001	*0.001	*0.001	*0.049
			2	*0.001	*0.001	*0.001	*0.032
			3	0.996	0.898	0.698	0.061
	TAMHANE	1	2	0.99	0.99	0.99	0.793
			3	*0.001	*0.002	0.027	*0.001
			4	*0.001	*0.001	*0.001	*0.049
		2	1	0.99	0.99	0.99	0.793
			3	*0.001	*0.003	*0.019	*0.001
			4	*0.001	*0.001	*0.001	*0.046
		3	1	*0.001	*0.002	*0.027	*0.001
			2	*0.001	*0.003	*0.019	*0.001
			4	0.996	0.954	0.902	0.064
		4	1	*0.001	*0.001	*0.001	*0.049
			2	*0.001	*0.001	*0.001	*0.046
			3	0.996	0.954	0.902	0.064

* The mean difference is significant at α=0.05 level.

Discussion

As insecticide intoxication has been declared to be an important public health problem throughout the world, many studies have been performed to examine the effects of these chemicals at both molecular and chromosomal levels. While these studies have generally included people professionally (vendors, sprayers, *etc.*) exposed to these agents [4–6, 9, 11–13], the study population of our study included ordinary people with indirect contact with insecticides. Additionally, while previous studies have examined people exposed to insecticides by respiratory or dermal routes, the population in our study consisted of people exposed by an oral route, *i.e.* by ingestion.

Intentional insecticide intoxication was found to be prevalent starting from adolescence (44.44%) mostly due to failing grades at school, abandonment by girl or boy friends, and especially matrimonial impediment; in adults (51.11%), the primary cause was generally matrimonial impediment, extreme incompatibility between mates, or financial depression.

Accidental intoxication from insecticides was primarily observed in children (52.38%), with ranges between 19.05% and 28.57% among older age groups, indicating that insecticides are unsafely used without necessary precautions, *e.g.* not keeping them away from children, in the Çukurova Region, with its abundance of agricultural fields resulting in a high frequency of insecticide use.

The values of AchE, SCE, MI, and RI were similarly affected by OP and CB group insecticides, but not by PY-group insecticides, in comparison with the control group. In particular, OP and CB insecticides are known to inhibit AchE when absorbed into the body, resulting in liver damage and death, if not tolerated by the organism [8, 19, 22]. The finding of similar significant decreases AchE levels in the case of OP and CB insecticide intoxication, in comparison with the control group, is concordant with other studies reporting the inhibition of these insecticides. OP and CB insecticides, received by an enteral route, spread to the whole body by circulation. These OP and CB compounds irreversibly bind to the AchE enzyme, and the result is the deposition of acetylcholine at all cholinergic junctions and synapses, with a resulting uninterrupted impulse conduction, hence paralysis. Synthetic pyrethroids affect the neuro-muscular system, but the reaction is short and rapid. This reaction is referred to as a sudden "knock-down" action. The extraction from the body is faster when compared to the OP or CB insecticides. Synthetic pyrethroids cause single or double breaks in the DNA, triggering cancer formation, while chromosomal breaks have not been reported [23]. Hence, the results of the PY group were similar to those of the control group.

A similar increase in SCE means in the OP and CB groups, but significantly higher increases than the PY and control groups, indicated the active intracellular penetration of these insecticides, increasing the SCE portion in the cells. Other studies have found significant SCE increases in the case of organic chlorinated OP and CB groups [16], with increases in SCE also being reported among farm workers who spray or are in contact with pesticides or herbicides and who are not using protective clothing [9, 12, 13]. A post-spraying increase in SCE among OP insecticide sprayers has been reported based on a study performed in our region [19].

The significant decrease in MI in patients exposed to insecticides by an oral route, in comparison to the control group, indicated a decrease in the cell numbers entering mitosis due to the ingested insecticides, in concordance with other studies [13, 24–26].

The decrease in RI and its proportionality to the exposure period of the farm worker has been reported [26]. But increases in RI in the case of enthrall exposure were found in our study, similar to another study of ours examining respiratory exposure [19]. But the MI decrease and RI increase explains the inhibition of cell division and replication by insecticides as well as the acceleration of the division cycle among stimulated cells. This explanation is also supported by our finding of more M_3-stage cells than M_2-stage cells. The significant increase in RI in all insecticide groups indicates that the small number of cells entering mitosis shortens the normal cell-cycle period, with the cells losing their division control, as chromosomal aberrations caused by insecticides can change the structure of functional genes acting in cell-cycle control.

In conclusion, insecticide exposure is generally intentional for the purpose of suicide in adolescents

and at older ages, but is generally accidental in children. Orally taken insecticides inhibit AchE, increase SCE, cause cell abnormalities, and impede the division and replication of cells as indicated by MI decreases with RI increases, while the speed of the division cycles in stimulated cells increases.

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