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Hematological and Biochemical Alterations in Occupationally Pesticides-Exposed Workers of Riyadh Municipality, Kingdom of Saudi Arabia

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Abstract: To assess the adverse health effects of pesticides on occupationally exposed workers, a cohort of pesticide sprayers, employed in Riyadh municipality, were interviewed and examined for changes in hematological profile, blood AChE activity, serum enzymes reflecting hepatotoxicity (AST, ALT and ALP) and markers of nephrotoxicity (urea and creatinine). There was a significant decrease in AChE activity ($p<0.001$) in pesticide workers ($n = 43$) relative to the control group ($n = 10$). No significant differences were detected in hematological parameters, except for WBC count which was significantly higher ($p<0.01$) in pesticides workers compared to the control group. Slight increases were observed in liver and kidney functions in the exposed group. The results indicated the need for official regulations and interventions enforced to reduce workers overexposure to pesticides throughout the Kingdom of Saudi Arabia.

Key words: Occupational exposure, insecticides, biomarkers, neurotoxicity, hepatotoxicity, nephrotoxicity

INTRODUCTION

In the last few years insecticides have extensively and still, been applied by Riyadh municipality to combat public health pests. Occupational exposure to pesticides causes many adverse health effects and several studies revealed that workers exposed to pesticides for prolonged periods are more likely to develop neurotoxicity, reproductive and developmental effects, immunological effects, leukemia, brain and prostrate cancers than the general population (Maroni and Fait, 1993; Hodgson and Levi, 1996; Zahm and Ward, 1998; Ojajarvi *et al*., 2000; Jenner, 2001; Mourad, 2005).

There is a good association between exposure to organophosphorus insecticides and inhibition of acetylcholinesterase (AChE) activity (Nigg and Knaak, 2000; Tapia *et al*., 2006) and therefore, the level of AChE activity has been considered a good biomarker for exposure to these pesticides (Remor *et al*., 2009; Joshaghani *et al*., 2007a; Eason and O'Halloran, 2002). Chronic exposures to pesticides also produce changes in blood parameters (Joshaghani *et al*., 2007b) and cause liver and kidney dysfunction (Ensberg *et al*., 1973; Hernández *et al*., 2006; Azmi *et al*., 2006; WHO, 1993, 1994).

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To the best of our knowledge, there was not nay attempt to study the adverse health effects of pesticides on pesticide workers in Saudi Arabia and because of the absence of official regulations for dealing with and handling pesticides, we have conducted this study to monitor some adverse health effects of pesticides on pesticide sprayers in Riyadh municipality.

MATERIALS AND METHODS

The study was conducted in Riyadh City, Kingdom of Saudi Arabia, from March to October, 2008, under grant No. 129-KSU-820. The sponsor of the project was King Saud University.

Participants Involved in the Study

A sample of 53 workers in Riyadh municipality (43 were pesticide applicators and 10 represented the control group who had no history of exposure to pesticides) were included in this study. The life work experience of participants was about 6.5±6.28 years. All workers responded to a questionnaire covering kind of insecticides they mostly used and health complaints they suffered from.

Hematological Analysis

Freshly blood samples collected from the arm vein were analyzed for hematological and biochemical assays. Total Red Blood Cell (RBC) count ($\times 10^6$ mm^{-3}), hemoglobin content (Hb) (g dL^{-1}), haematocrit (HCT) (%), total number of WBCs (leukocytes) ($\times 10^3$ mm^{-3}) and their differential count were assessed. Mean Corpuscular Volume (MCV) (fl), Mean Corpuscular Hemoglobin (MCH) (pg), MCH Concentration (MCHC) (g dL^{-1}) and red blood cell distribution width (RDW) (%) were also calculated from the data obtained. Platelet count and parameters related to platelets ($\times 10^3$ mm^{-3}), plateletcrit (PCT) (%), Mean Platelet Volume (MPV) (fl) and Platelet Distribution Width (PDW) (%), were calculated.

Biochemical Analysis

Levels of alanine aminotransferase (ALT, EC 2.6.1.2), aspartate aminotransferase (AST, EC 2.6.1.1),), alkaline phosphatase (ALP, EC 3.1.3.1), acetylcholinesterase (AChE, E.C. 3.1.1.7), urea and creatinine were checked in blood samples. These parameters, except AChE, were spectrophotometrically analyzed using biochemical kits from Chematil, Italy. Acetylcholinesterase activity was measured according to Pickering and Martin (1970). The level of ALT, AST and ALP was expressed as Unit per liter (U L^{-1}), while urea and creatinine concentrations as milligram per deciliter (mg dL^{-1}). Change in pH per hour (Δ pH h^{-1}) was the measurement unit of AChE activity.

Statistical Analysis

Significant differences between mean values of exposed and control groups were statistically analyzed using the Student's t-test, utilizing the computer program, Sigmaplot for windows (version 2.0).

RESULTS

Table 1 shows the pesticides used by Riyadh municipality to control public health pests. Organophosphates and pyrethroids represented 80% of the used insecticides. Temephos, was the most often used insecticide (95.3%).

Table 1: Pesticides frequently used by the workers

Pesticide	Group	WHO category	N = 43	%
Temephos	Organophosphate	III	41	95.3
Permethrin	Pyrethroid	II	30	69.8
Diazinon	Organophosphate	II	29	67.4
Fenitrothion	Organophosphate	II	28	65.1
Deltamethrin	Pyrethroid	II	27	62.8
Lamda-cyhalothrin	Pyrethroid	II	25	58.1
Bacillus thuringensis	Microbial	III	19	44.1
Primiphos-ethyl	Organophosphate	Ib	19	44.1
Alpha-cypermethrin	Pyrethroid	II	19	44.1
Chlorpyrifos	Organophosphate	II	18	41.9
Fenvalerate	Pyrethroid	II	18	41.9
Bioallethrin	Pyrethroid	II	17	39.5
Pyriproxyfen	IGR	U	15	34.9
Diflubenzuron	IGR	U	15	34.9
Malathion	Organophosphate	III	10	23.2

WHO (2005) classification: Ib = Highly hazardous, II = Moderately hazardous, III = Slightly hazardous, U = Unlikely to pose acute hazard in normal use

Table 2: Health complaints of workers

Complaint	N = 43	%
Skin itching	26	60.5
Headache	25	58.1
Coughing	24	55.8
Sweating	18	41.9
Difficult breathing	15	34.9
Fatigue	15	34.9
Blurring of vision	15	34.9
Dizziness	14	32.5
Changes in mood	14	32.5
Sleeplessness	13	30.2
Vomiting	13	30.2
Forgetfulness, memory disorders	12	27.9

Health complaints of workers, possibly related to insecticides exposure are shown in Table 2. Skin itching, headache and coughing were the most common symptoms (60.5, 58.1 and 55.8%, respectively); lower proportions of workers complained of sweating (41.9%), difficult breathing, fatigue and blurring of vision (34.9%), dizziness and changes in mood (32.5%), sleeplessness and vomiting (30.2%) and workers suffering from forgetfulness or memory disorders represented 27.9%.

No significant differences were found between hematological parameters in pesticides-exposed and control groups (Table 3). However, a significant increase in WBC count ($p<0.01$) was observed in the pesticide-exposed group and significant increases in lymphocytes and monocytes counts ($p<0.01$) were recorded in this group.

A highly significant difference ($p<0.01$) was found between AChE activity in pesticide workers and control group (Fig. 1), the enzyme activity in pesticide workers was lower than its activity in control group by 31.5%.

Serum biochemical parameters of pesticides sprayers exposed and control subjects are shown in Table 4. A slight increase, but not statistically significant, was observed in all tested parameters in pesticide workers compared to control subjects.

The levels of AST and ALT in pesticide workers were higher than levels in control group by 22.3 and 16.8%, respectively. ALT level was higher than the upper threshold value in 5 workers, whereas AST level was higher than the threshold value in two workers only.

The increases in urea and creatinine concentrations in pesticide workers were 16.1 and 5.7 %, respectively.

Table 3: Hematological profile in study groups

	Workers (n = 43)		Control (n = 10)		
Parameters	Mean±SE	Max.-Min.	Mean±SE	Max.-Min.	p-value
RBC ($\times10^6$ mm^{-3})	5.48±0.08	6.73-4.52	5.24±0.20	6.28-4.34	0.23
Hb (g dL^{-1})	15.13±0.25	18.4-8.4	14.97±0.39	16.8-13.3	0.76
HCT (%)	45.28±0.60	56.2-31.6	44.54±0.85	49.7-40.6	0.58
MCV (fl)	82.77±1.06	90.8-56.7	83.12±2.29	93.3-71	0.89
MCH (pg)	27.85±0.44	31-15.1	27.29±1.01	30.6-21.2	0.59
MCHC (g dL^{-1})	33.41±0.23	36.2-26.6	32.97±0.49	35.1-29.8	0.41
RDW (%)	13.88±0.28	23.8-12.0	13.57±0.26	15.2-12.3	0.61
PLT ($\times10^3$ mm^{-3})	262.20±11.2	568-146	218.50±11.3	252-158	0.07
PCT (%)	0.25±0.09	0.406-0.112	0.25±0.02	0.413-0.204	0.99
MPV (fl)	10.39±0.16	12.8-8	10.02±0.37	12.1-8.4	0.32
PDW (%)	13.35±0.34	18.9-9.6	13.10±0.77	17.9-9.9	0.75
WBC ($\times10^3$ mm^{-3})	10.36±0.41**	16.4-5.5	7.71±0.56	10.5-5.5	0.005
LYM ($\times10^3$ mm^{-3})	4.18±1.14**	7-2.2	3.04±0.58	2.2-4.4	0.004
MON ($\times10^3$ mm^{-3})	1.08±0.05**	2-0.5	0.73±0.07	1.2-0.5	0.006
GRA ($\times10^3$ mm^{-3})	5.02±0.27	9.8-2	4.31±0.32	5.4-2.3	0.23

**: Moderately significant (p<0.01) according to Student t-test

Table 4: Biomarkers of liver and kidney functions

	Workers (n = 43)		Control (n = 10)		
Parameters	Mean±SE	Max.-Min.	Mean±SE	Max.-Min.	p-value
AST (U L^{-1})	28.42±1.58	68.5-15.4	23.24±1.70	30-13.2	0.13
ALT (U L^{-1})	33.64±2.95	115.6-13.6	28.80±2.20	47.5-13.9	0.44
ALP (U L^{-1})	187.60±7.58	336-94	177.00±20.22	282-119	0.57
Urea (mg dL^{-1})	34.43±1.50	87.2-21.6	29.64±1.65	39.2-20.4	0.14
Creatinine (mg dL^{-1})	0.93±0.04	2.2-0.6	0.88±0.03	1-0.7	0.51

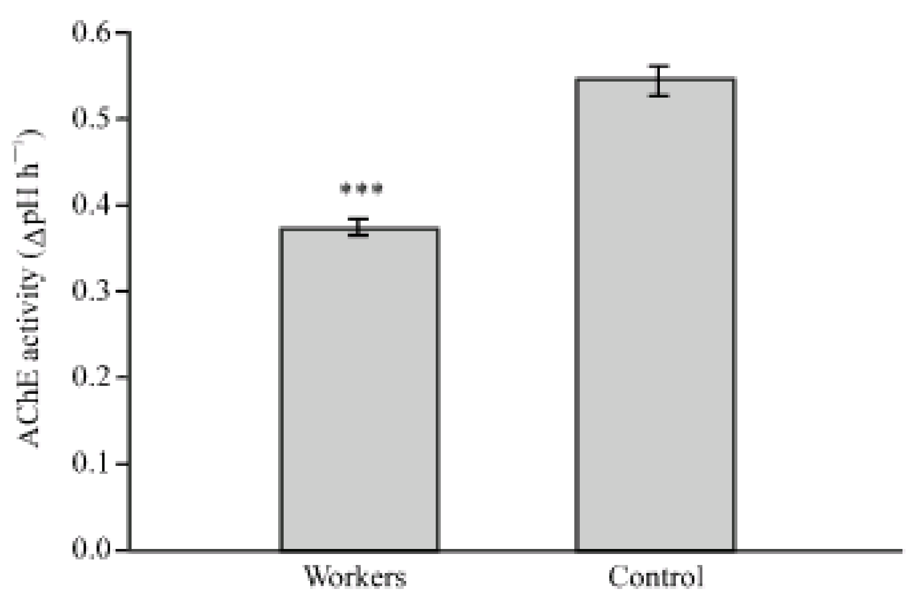

Fig. 1: Mean of blood AChE activity in pesticides-exposed workers (n = 43) compared to control subjects (n = 10). ***Difference is highly significant (p<0.001) using Student's t-test

DISCUSSION

Pesticide sprayers in Riyadh municipality are exposed to different classes of insecticides during their work. The most frequently used insecticides (organophsphorous esters and pyrethroids) have neurotoxic actions. Workers reported having complaints of skin itching, headache, coughing, sweating, dizziness, fatigue, vomiting and vision, respiratory and behavioral problems which are common among people dealing with pesticides (Kishi *et al*., 1995; Strong *et al*., 2004). Del Prado-Lu (2007) reported that headache was the most frequently symptom (48%), followed by easy fatigability (46.1%) and cough (40.2%) in cutflower farmers exposed to pesticides.

No significant differences were found between control and exposed groups for most hematological parameters; similar results were reported by Pastor *et al*. (2002), Lebailly *et al*. (2003) and Remor *et al*. (2009). However, some studies reported that occupational exposure to pesticides resulted in alterations of hematological parameters (Amr, 1999; Mourad, 2005). This contradiction may be attributed to the types of pesticides used and exposure conditions. The increase in WBC count in the present study was associated with a significant increase in lymphocytes and monocytes count, an increase in WBC count in agriculture workers was also reported by Jamil *et al*. (2007).

Temephos was the most frequently insecticide applied by sprayers in Riyadh municipality, the inhibition of AChE activity in the exposed group could be attributed to their exposure to this organophosphorus insecticide. Our results support the previous findings of Vidyasagar *et al*. (2004) and Hernández *et al*. (2005), who reported significant reductions in AChE activity in pesticides exposed subjects. RBC cholinesterase inhibition has been frequently used to establish safe exposure levels or reference doses (RfDs) for organophosphate pesticides (US Environmental Protection Agency, 1997). In the state of Washington, blood ChEs of mixers, loaders and applicators are monitored by a single state laboratory; a 20% or greater depression of ChEs leads to investigation of the worksite and a depression of 30% or greater of RBC AChE, or 40% of plasma ChE requires removal of workers from the field (Keifer, 2005). The reduction of AChE activity by 31.5% in pesticide workers in the present study necessitates that official regulations and interventions should be applied to protect workers dealing with pesticides from the adverse health effects of these chemicals.

The increase in the level of ALT and/or AST is a good indicator of hepatic toxicity (Hall, 2001). The present study showed a slight increase in AST, ALT and ALP levels (Table 4). Significant increases in the levels of these enzymes were found in occupationally exposed workers (Tomei *et al*., 1998; Khan *et al*., 2008).

Liver dysfunction with raised AST and ALT was observed in agriculture workers in India (Patil *et al*., 2003) and in selected farm workers in Gadap Karachi, Pakistan (Azmi *et al*., 2006). Changes in AST levels in agriculture workers was related to their exposure to pesticides (Hernández *et al*., 2006).

The present study revealed insignificant elevated levels of serum urea and creatinine among pesticide sprayers. Previous studies have reported subtle nephrotoxic changes in workers occupationally exposed to pesticides with higher levels of creatinine and urea (Al-Qarawi and Adam, 2003; Attia, 2006). Researchers exposed to pesticides in a chemical plant showed elevated creatinine concentration which supports the subclinical kidney impairment (Kossmann *et al*., 2001).

In summary, biomarkers tested in this work, especially AChE level, might be used to detect early hematological and biochemical effects of pesticides to remove workers from workplace before having adverse clinical health effects. The results of the present study indicate the need for official regulations and interventions to reduce workers' overexposure to pesticides throughout the Kingdom of Saudi Arabia.

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