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Research Article

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Assessment of cholinesterase in greenhouse workers exposed to insecticides in the coastal region of Syria

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ABSTRACT

To evaluate the health impact of spraying anticholinesterase insecticides (organophosphates and carbamates)100 sprayers in the greenhouses of coastal region in Syria were selected randomly. Serum butyryl cholinesterase (BChE) was assessed among sprayers after spraying pesticides and the findings obtained were compared with those determined in a reference group(n=50).The most common symptoms observed were itching/skin irritation (34%) and eye irritation and ophthalmia(17%) in the exposed workers. Serum butyrylcholinesterase (BChE) was significantly decreased in workers(P<0.01). Monitoring of BChE in pesticide sprayers in greenhouses could be useful to predict and prevent health hazards ofthese pesticides.

Key words: Greenhouses, anticholinesterase insecticides, butyryl cholinesterase

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INTRODUCTION

Greenhouses are microcosms aimed at providing physical environments suitable for increase growth and production of crops and pesticide application is a common practice to improve quality and quantity of agricultural products. Unfortunately, the enclosed conditions makes sure that greenhouse workers are more exposed to higher levels of plant protection products than general agricultural workers[1]. Pesticides widespread in agriculture sector are most economical approach to control the insects and pests, though are major contaminants of our environment and highly toxic to non-target organisms. The spray-workers during sprays on crops are directly exposed to pesticides while mixing, handling, spray, and through contaminated soil, air, drinking water, eating food and smoking at work places[2]. Most occupational exposures to pesticides occur from skin absorption, although inhalation may be an important route of exposure during pesticide manufacture and application; the gastrointestinal tract and eyes may also be affected[3]. Pesticide exposure of greenhouse workers has been determined to be at a level where health risks may occur[4], and especially dermal exposure after reentry to a pesticide treated greenhouse, with manual handling of plants is associated with high transfer of pesticides[5]. There are strong associations between exposures to aerial pesticides and symptoms and cholinesterase is significantly reduced in exposed populations[6].Even though organophosphorus (OP) and carbamate (CB) pesticides are considered neurotoxic to humans[7], they are two classes of widely used pesticides [8, 9]. According to He et al [10], these chemicals are predominantly used in developing countries. The OP pesticide class consists of the esters, amides, and thiol derivatives of phosphoric, phosphonic, or phosphinic acid[11]. These compounds are mainly used as insecticides, since they act on the nervous system of insects, but they can also be part of some herbicides and fungicides[12]. CB are structurally less complex, derived from the esters of carbamic acid (NH3CO2), and generally used as fungicides, herbicides, or insecticides[7]. OP and CB are known to act as neurotoxic agents, as they can inhibit the activity of cholinesterases (ChE) such asacetylcholinesterase (AChE) and butyrylcholinesterase (BChE). The interference with the activity of the neurotransmitter acetylcholine(ACh) may cause both short- and long-term effects[11, 13, 14].Regarding a classification of OP, the common denominator is the presence of a phosphorus atom, either in a phosphoryl (P=O) or thiophosphoryl (P=S) group[15]. These chemical compounds act via different mechanisms: while some may interfere directly (without metabolic or biotransformation) with ChE, others act through indirect inhibition, i.e. they need metabolic transformations in order to exert inhibitory activity towards these enzymes[11, 15]. In the latter case, especially OP with a P=S moiety are exposed to various biotransformations after absorption, which may significantly change their toxicity characteristics, resulting in the formation of metabolites which are sometimes more toxic than the original substance[16]. Especially so, when the metabolic activation proceeds via enzymes of the cytochrome P450 complex, which results in the formation of oxygen analogues of pesticides (oxons), which in turn inhibit AChE and BChE [15]. Furthermore, reversible and irreversible complexes can be formed. OP usually form more stable complexes – sometimes even irreversible - with AChE and BChE, whereas CB tend to form less stable and reversible complexes[17, 18]. Evidently, the individual effects of OP or CB poisoning are determined by the metabolism, and accordingly related to the individual chemical structure[16].Analytically, AChE depression of 15% compared with baseline indicates overexposure [15], 40–50% depression is associated with mild neurotoxic effects and serious effects can occur at 80% depression[19].

The typical human exposure assessment approach is to measure metabolic biomarkers in blood. No study was reported in coastal region of Syria on exposed workers previously, therefore this study was conducted with the aim of evaluating impact on health produced by use of anticholinesterase insecticides in greenhouses.

EXPERIMENTAL SECTION

The study was cross-sectional in design.

Subject Selection and Blood Sample Collection: The protocol was approved by Ethical Committee of Damascus University, Faculty of Pharmacy and informed consent was obtained. Greenhouses were identified in coastal region of Syria. The blood samples were collected from 100 greenhouse workers (aged 18-61 years, mean ±SD; 34.5±10 years), exposed to OP and CB pesticides and 50 control subjects (aged 19-58 years, mean ±SD; 35.3 ±10.6 years, belonging to similar socio-economic status and never involved either in agricultural operations or pesticide handling). The blood samples (5 mL) were collected into dry tubes(without anticoagulant). Structured questionnaires were used to collect information.

Serum Cholinesterase Activity: Serum was separated from blood by centrifugation at 2000-3000 r.p.m for 15 min at ambient temperature. Samples were stored at –20 °C until assay of ChE activity(done during 48 hours after collection). The BChE activities were measured according to the procedure of Ellman et al.[20]based on the colorimetric estimation of unreacted acetylcholine using special kit (Greiner, DGKC-94, Germany). The reference range for human serum ChE activity is 3.93-10.80 kU/L for women and 4.62 -11 kU/L for men.

Statistical analysis

Enzyme activities and serum total protein levels were expressed as mean ± standard deviation of mean (SD). Differences between group means were detected by analysis of variance, Student's t-test and Pearson Correlation Coefficient. Data were analyzed with the SPSS 13.0 statistical package. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

The demographic characteristics of the two groups are presented in Table 1. The most common pesticides were used anticholinesterase insecticides like methomyl, dimethoate, carbaryl, carbosulfan, chloropyrifos, profeniphos, indoxacarb, oxamyl, fenamiphos, etc.

Table 1: Characteristics of Participants

Variable	Greenhouse workers (n = 100)	Controls (n = 50)
Mean age (years)	34.5	35.3
Sex	95 males 5 females	48 males 2 females
Smoking habit	77%	68%
Education	low level	low level

The level of knowledge among the farmers is seen from Table 2, where answers about factors with a possible influence on intoxication of humans when handling pesticides are listed.

Table 2: Factors of importance for intoxications in humans when handling pesticides (n = 100)

Factor	greenhouse workers (n= 100) Yes	No
Using protective means		
Gloves	0	100
Mask	3	97
Plastic poncho	0	100
Reading and following instructions on labels of pesticide packages	0	100
Smoking during and after applying pesticides	47.4%	52.6%
Eating and drinking after applying pesticides	0	100
Washing after handling pesticides	100	0

The most frequent symptoms mentioned were itching/skin irritation (34%) eye irritation and ophthalmia(17%),Table 3. The experience of symptoms in connection with spraying was found to be depending on the degree of pesticide exposure.

Table 3: Symptoms

Symptoms	Number	%
No symptoms	64	64
itching / skin irritation	34	34
eye irritation and ophthalmia	17	17
sneeze	10	10
shortness of breath	7	7
lacrimation	6	6
Vomiting	6	6
nausea	3	3
throat sore	2	2
muscular spasm	1	1
neurological pain	1	1
cough	1	1
headache	1	1

The mean ChE activity was found to be depending on the degree of pesticide exposure as seen in Table 4.

Table4: Serum butyryl cholinesterase (BChE) values

	Greenhouse workers(n=100) mean±SD	Controls(n=50) mean±SD	P value
BChE	7218.82±1787.82	8841.14±1068.60	0.000*

** Significantly different from control (p < 0.01)*

No statistically significant differences were found in the exposed group regarding age, symptoms and smoking, while there was a statistically significant difference in BChE values regarding smoking or not after applying pesticides, Table5.

Table 5: BChE values regarding smoking or not after applying pesticides

	Smokers in exposed group(n=76)		
	Nosmoking after applying pesticides n=40 mean±SD	smoking after applying pesticides n=36 mean±SD	P value
BChE	7476.05±1807.08	6467.19±1494.20	0.010*

** Significantly difference ($p < 0.05$)*

In undertaking this kind of study, we must first consider the difficulty of quantification and assessment of the effects of the exposure that may lead to chronic intoxication. Multiple exposures of different pesticides which might interact in an additive or multiplicative way and so could affect the pattern of health effects expected in the case of mono-exposures. The other important points that have also affected this study include the large number of spraying made and the usage of various pesticides during the period of study, as well as the lack of “baseline” data for the cohort of individuals exposed to pesticides in greenhouses. Despite all these limitations, it is possible to draw conclusions about the overall health risks of these complex exposures.

BChE activities are indicators of exposure to OP pesticides and carbamates and widely used to determine the rate of exposure[21].

Our study demonstrated significantly lower BChE activity in pesticide spraying workers in greenhouses compared to controls ($p < 0.01$). This has been reported in several studies [22-24]. The observation of lowered BChE activity in greenhouse farmers at coastal region of Syria is probably due to unsafe working habits promoted by self-desires to have rapid knockdown of pests and increase income. The knowledge of how to handle pesticides and the use of protective measures were poor in the actual study, as seen from Table 2, and also found in earlier studies[25, 26].

Several studies on volunteers reported that repeated long term exposures of organophosphorus pesticide and carbamate decrease the blood cholinesterase activities without clinical manifestation[27]. The associations of symptoms and ChE levels with a higher frequency of pesticide use could reflect a cumulative effect of repeated exposure, but it could also be explained simply by the fact that people who have used pesticides more often have had more opportunity to develop acute symptoms and/or a lowered ChE level. The time interval between spraying and the blood test taken might also explain the lack of significant correlation between symptoms and serum ChE levels, although the farmers without symptoms did have a higher mean level. A better indicator would have been red blood cell cholinesterase activity as a marker of biological effect, whereas serum ChE is a marker of exposure[28].

CONCLUSION

Serum butyrylcholinesterase (BChE) were significantly inhibited due to multiple exposure to different pesticides. Mixing of pesticides before spraying and direct reentry soon after spraying play significant roles in reducing serum butyryl- cholinesterase activity. Occupational exposure to pesticides may be monitored by the measurement of the activity of ChE enzymes in order to predict the risk of pesticide poisoning. For preventing the toxicity, required safety precautions can be taken in advance.

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