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Cholinesterase Activity Among Spray Workers in Iran

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Abstract: In this study whole blood cholinesterase activities of the agriculture and hygiene spray workers exposed to organophosphorus and carbamate compounds from different parts of Tehran Province in Iran were determined. Lovi Bond method was used in three stages including prepare the questionnaires about spray worker body health conditions, taking their blood samples before and after working and their insight to insecticides and prepare the required solutions. Results showed that no any changes were observed in the 50% of the spray worker blood cholinesterase activity after working. In the 32.4% of them, cholinesterase activity has decreased up to extensive poisoning and in the 17.6% of them cholinesterase activity was much decreased at the end of an acute or severe poisoning, whereas in testifier workers less than 5.9% of them cholinesterase activity was decreased and in the 94.1% of testifier workers cholinesterase activity was normal. Analysis of the data demonstrated that no significant relationship between spray worker blood cholinesterase activity, age groups, history of working, knowledge, cigarette smoking, history of toxicity and their responsibilities were observed. The measurement of pre-exposure cholinesterase values is essential for comparison of the values after pesticide application.

Key words: Poisoning, phosphorous, carbamate, cholinesterase blood activity, spray workers

INTRODUCTION

Organic insecticides have been used to control insects from 1939 which DDT insecticidal properties were discovered by Paul Muller (WHO) and their effectiveness against different insect species is accompanied by the disadvantage of high mammalian toxicity. The widespread use of organophosphorus compounds (OPs) as pesticides and the frequent misuse of OP nerve agents in military conflicts or terrorist attacks emphasize the high clinical relevance of OP poisoning (Aurbek *et al*., 2009) such as self-poisoning (Sam *et al*., 2009) and long-term influence of poisoning on depressive symptoms (Beseler and Stallones, 2008) and easy accessibility to these compounds results in a huge number of intoxications. In addition, accidental exposure is the main cause for mild poisoning and severe cases are mostly because of suicidal use (Stefanidou *et al*., 2009). The World Health Organization (WHO) and the United Nations Environmental Program estimate that one to five million cases of pesticide poisoning occur among agricultural workers each year with about 20000 fatalities, the majority occurring in developing countries (Rosenstock *et al*., 1991; Pimental *et al*., 1992). The true incidence of poisoning is likely to be considerably higher. Since the estimate is based on self-reported cases (Jeyaratnam *et al*., 1987) or on inference from vital statistics (Loevinsohn, 1987).

Organophosphorus Pesticides (OP) and Carbamates (CB) are two classes of widely used pesticides (Reimer *et al*., 2007; Bosgra *et al*., 2009). These toxic compounds can be absorbed through the skin, mucous membranes, gastrointestinal and the respiratory tracts and produce their toxic effects by the inhibition of acetylcholinesterase (AChE) and the subsequent accumulation of synaptic acetylcholine (ACh) in peripheral and central nervous systems (Karczmar, 1998; Tafuri and Roberts, 1987).

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In Iran, phosphorous and carbamate insecticides have been used for agricultural pests and vector borne diseases control, for many decades. Human poisoning due to these insecticides may occur by exposing to insecticides, during chemical tools and dish washing, drinking of contaminated water and foodstuff eating. These compounds have physical and chemical characteristics with different degree of toxicity for mammalian and other creatures. They can cause human poisoning and lead to an acute or a chronic disease such as mild or severe disorders in the cholinesterase enzyme function and consequence blocking the neurons activities by disrupting of cholinesterase enzyme function, which ending to death finally (Coye *et al*., 1986).

The acute toxicity of organophosphorus insecticides (OP) has been predicted by their action on red blood cell acetylcholinesterase (RBC-AChE) (Eyer *et al*., 2007). In addition, OP covalently binds to other serine esterases, primarily butyrylcholinesterase (BuChE) (Debord *et al*., 2008). According to current knowledge, inhibition of acetylcholinesterase (AChE) is a very important toxic action of organophosphorus compounds (OP). Hence, it is obvious to follow the AChE activity in order to quantify the degree of inhibition and to assess possible reactivation. Red Blood Cell (RBC)-AChE provides an easily accessible source to follow the AChE status also in humans (Eyer *et al*., 2007). Enzyme activities in body fluids are often utilized as diagnostic markers for physiological conditions and diseases (Kao and Gratzl, 2009). So, the measurement of cholinesterase enzyme function in spray workers especially carbamates and phosphorus compounds is a routine assessment which should be measured. Poisoning recognition and treatment are being important for physicians. In this study the activity of cholinesterase enzyme in spray workers was measured by Lovi Bond method because a little blood volume is required. It should be mentioned that some of these workers work in some seasons and there is no controlling on their activities. So, temperature of the environment, time of the usage, rate of the work in a day, lack of the information about the consuming insecticide hygienic dangers, literacy and using of the protective tools are the factors which would be studied.

MATERIALS AND METHODS

The study was carried out in Tehran Province from different parts of the agriculture and hygiene during 2006. Attention to wide simultaneously using of insecticide groups and limitation of spray worker poisoning, we communicated with many companies after several counsellorships. From 142 persons of the different parts of the agriculture and hygiene that selected, 34 persons as testifiers and 108 persons as spray workers. The testifier persons were selected randomly among people who have not any spraying activity from 6 months ago.

The Elman method which named also as Lovi Bond method was used because a little blood is required for tests (Elman *et al*., 1961). This method has a minimum cost and in any condition is practicable (Elman *et al*., 1961; Jeyaratnam *et al*., 1986). Examinations were done in three stages. At the first stage, the questionnaires were prepared to evaluate the spray workers body health conditions and their insight to insecticides. At the second stage, solutions like BTB (Bromo Thymol Blue) and acetyl choline perchlorate as substrate, distilled water without CO_2, tools for compressing Lovi Bond and timer were prepared. For confirming materials validity, we prepared first 0.5 mL from BTB and 0.01 mL from blood of a person as testifier who had not contact to any insecticides and 0.5 mL from substrate solution were obtained in Lovi Bond machine. Another sample was obtained from testifier blood with water without CO_2 were placed in the left part of the machine, then in front of the light the disk were circled until the color of filter was as like as the solution. If the changes more than 12.5% were seen, it should be done again, because a little changes in pH, causes disorders in the results of experiments. After they were sure from preparing the introducers, the experiments were done on spray workers.

At the third stage the blood sample of the spray workers were taken before and after the work. Before the spray workers starting to work, blood samples were taken. An experimental pipe was allocated to any spray worker and the specification and his number similar to the questionnaire number which was noted on them. Workers should have washed their hands with water and soap. In each pipe was poured 0.5 mL from BTB introducer and 0.01 mL blood from finger-tip of each spray worker was taken which added to BTB solution. This action was done for testifier pipe too. Then 0.5 mL from substrate was poured to the pipes and the time was noted, as soon as 1 min passed, testifiers samples were added and placed in the right part of the Lovi Bond compressor machine. Solution colour alteration in the control pipe was showed that experiment was occurring. Spray worker's blood colour alteration after 1 min was showed that experiment was occurring and consequence the amount of cholinesterase enzyme activity. Significant differences between variables were analyzed by one-way ANOVA using SPSS program.

RESULTS

In this study, 142 persons in Tehran Province from different parts of the agriculture and hygiene were examined. From 142 persons, 34 persons as testifiers and 108 persons as spray workers were considered. The testifier persons were selected randomly among people that have not any spraying activity from 6 months ago. From 108 spray workers, 91 persons have been working with phosphorous pesticides and 17 persons have been working with carbamate pesticides. Spray workers have between 20-60 years old. The 31.5% of them were in the 31-40 years old group.

From the whole of 108 spray workers in this study, 72 person (66.7%) were as sprayers, 18 persons (16.7%) as solution makers, 7 persons (6.5%) as head workmen and others (10.2%) were in contact to pesticides in any way (Table 1). From education, 52.8% had primary school, 10.2% had ability to read and write and 25.2% had higher education. Among higher education, 3 persons had university education that were used as head workmen in the hygiene part. From history of working, most of them have been working for more than 10 years (44.5% of them) with different kinds of pesticides and 13.9% of them have been working with these compounds less than 1 year.

Because of the time and contact with insecticides are very important, 97.2% of them have been working more than 5 h day^{-1} and the risk of toxicity are higher in them (Table 1). From the spray worker knowledge about insecticides and mode of action, 48.2% of them even don't know the name of the insecticide that they have been working with it. The 35.2% of them have no knowledge about the activity of toxins and way of the causing toxicity; just 16.7% of them have a little knowledge about insecticide effects and activities (Table 1).

About the history of toxicity with insecticides, 37.9% of them have been poisoned in different ways before. From spray worker who used personnel protective tools, 38.3% of them, used all protective tools, 28.9, 1.6 and 20.3% of them used protective cloths, gloves and mask alone, respectively and 10.9% of them didn't used any protective tools, while they have been working with insecticides (Fig. 1).

From 108 of the spray workers, 40 persons (37%) of them had no problem in that time and 68 people (63%) proclaimed different diseases. Among these 68 persons, 18 persons of them had only one problem and 50 persons of them complained from more than one problem that most of them were headache, apnea and vertigo. Also, 55.5% of those who work with carbamate complained from other problems like muscle weakness, tearing, saliva increasing, dimness of sight, whereas, testifier workers were complained a rare problem like tiredness, apnea and muscle weakness. Workers who had contact with toxin were more complaining than testifier workers. Apnea and vertigo were signs that most of the workers were complained from them.

In this study, two blood samples were taken from spray workers before and after the daily work. Results showed that no changes were observed in the 50% of the

Table 1: Results from the spray workers variables according to taken information

	Frequency	
Variables	No.	Percent
Age group	33	30.6
21-30	34	31.5
31-40	28	25.9
41-50	13	12.0
51-60		
Occupation		
Sprayer	72	66.7
Solvent maker	18	16.7
Head workman	7	6.5
Other	11	10.2
Education		
Illiterate	12	11.1
Ability to read and write	11	10.2
Primary school	57	52.8
Higher	28	25.9
Work history (year)		
<1	15	13.9
2-5	14	13.0
6-10	31	28.7
>10	48	44.5
Daily work (h)		
1-3	1	0.9
3-5	2	1.8
>5	105	97.2
Knowledge		
Good	18	16.7
Little	38	35.2
No	52	48.2
Cigarette smoking history		
Yes	39	36.1
No	69	63.9
Cigarette smoking during working		
Yes	25	64.1
No	14	35.9

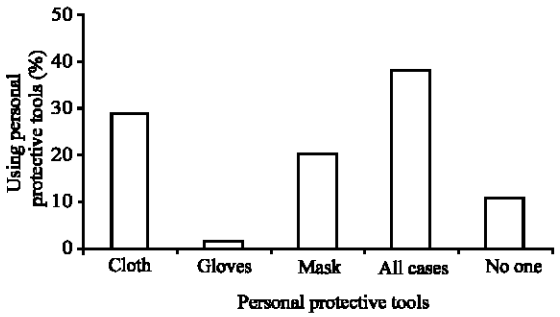

Fig. 1: Distribution of spray workers according to using personnel protective tools

Table 2: Distribution of spray workers according to blood cholinesterase activity

	Blood cholinesterase activity*				
Result test	0-25	26-50	51-75	76-100	Total
Morning					
No.	0.0	5.0	34.0	69.0	108
%	0.0	4.6	31.5	63.9	100
Afternoon					
No.	2.0	14.0	32.0	60.0	108
%	1.9	13.0	29.6	55.6	100
Total result					
No.	0.0	19.0	35.0	54.0	108
%	0.0	17.6	32.4	50.0	100
Control					
No.	0.0	0.0	2.0	32.0	34
%	0.0	0.0	5.9	94.1	100

*Individuals that blood cholinesterase are 0-25%: Sever poisoning, 26-50%: Weak poisoning, 51-75%: Poisoning ready and 0-25%: Normal

spray worker blood cholinesterase activity after working. In the 32.4% of them, cholinesterase activity has decreased to the end stage as an extensive poisoning and in the 17.6% of them cholinesterase activity was decreased very much that they were poisoned very acute and severe, whereas in the testifier workers less than 5.9% of them cholinesterase activity was decreased a little and in the 94.1% of testifier workers cholinesterase activity was normal (Table 2).

DISCUSSION

Cholinesterase is one of the essential enzymes for especial functions of human neurological systems. Some of the pesticides groups like organophosphorus and carbamates have interfered in cholinesterase activity and have blocked it. These compounds can be poisoning for human under particular conditions. The effects of this compound can result from breathing, eating, or contact with skin and mucous during the stages of working, preparing of the solutions, spraying, or warehousing and etc.

In the organophosphorus compounds, insecticides like malathion, diazinon, etc. and in the carbamates compounds insecticides like carbaryl and propoxur are most used as an insecticide in agriculture and hygiene pest control. It should be mentioned that some of these workers work in some seasons and there is no controlling on their cholinesterase activities.

In this study, the decrease of cholinesterase enzyme activity showed that 50% of these spray workers were at moderate risk of toxicity (32.4%) and mild risk of toxicity (17.6%), whereas blood cholinesterase activity in testifier workers was normal in the 94.1% of the persons and in the 5.9% of them, cholinesterase enzyme activity was decreased between 50-75%. So, the difference between the level of cholinesterase enzyme activity in spray workers and testifiers was significant. These finding showed that the measurement of cholinesterase enzyme function in spray workers especially carbamates and phosphorus compounds is a routine blood exam test which would be measured. As working with toxins should be done by healthy people, young people are usually chosen for this reason in different countries (Mekonnen and Agonafir, 2002). So, in this study the spray worker age was important and most of them were in the 30-40 year age groups that were suitable for this purpose.

Spraying is usually done by low educated or illiterate people, especially in agriculture part. In this study, we observed that, although most of spray workers are illiterate, but a few of them have elementary education or even university education.

As toxins are absorbed from skin and membrane tissues, eating, drinking and smoking during the work with the toxins increases the toxin absorption, so during working with the pesticides, spray workers should not be do these works in any way. After the filling of the questionnaires form by the spray workers, we observed that they have avoided from the eating and drinking liquids during the work, but one third of them were addicted to cigarette smoking (it means that they smoked 5 cigarettes at least per day) and more than half of them had no notice to side effects of smoking that was due to ignorance or sever addiction of them to cigarette.

In workers, those who always work with toxins, if they had no knowledge about protective tools, the possibility of toxicity is higher. For this reason, history of the spray workers toxicity showed, that among of them, the 38% of them would have toxicity history. Apnea, nausea, vomiting and vertigo were symptoms which they were complained from them and most of them had more than one symptom.

In most countries, spray workers work in some hours of the day and they are not in the exposure of toxins in all of the day (Mekonnen and Agonafir, 2002). Although, in this study, most of spray workers work 5 h per a day but they work in some season and this was an important factor to return the cholinesterase level to the normal rates.

Most of the spray workers and the other people who are in the exposure to the toxins had no knowledge about hygienic risks. Toxin packages have the warning labels but spray workers usually have no notice to them (Mekonnen and Agonafir, 2002). Among spray workers only 16.7% of them had enough knowledge about toxin mode of actions, side effects and protective ways and tools which would be used against them. According to these, educating of chief workers about toxins and side effects is more important because they have higher

education and are usually fixed, so they could teach their workers, to lesson the side effects of the pesticides.

One of the effective ways to decrease the side effects of the toxins is using the personal protective tools that cause decreasing the contacts and breathing of the insecticides. We should be noticed that personal protective tools of the spray workers should be well and have no tear on it, because using the torn personal protective tools, not only have no protective effect on persons, but also may cause more toxicity of them. Using the personal protective tools depends on kinds of the spray worker actions, kinds of the toxins and its formulations. Usually workers who work in extensive parts used personnel protective tools, whereas who work in a house or a small parts no pay attention to this subject. The reasons that spray workers don't use the personnel protective tools which these are not being suitable, causing allergy, apnea, warming and even not being effective.

According to studies have been done by Mekonnen and Agonafir (2002), after suitable educations, workers are more willing to using protective tools. In this study, only about 38.3% of the spray workers used personnel protective tools completely that there was significant differences between cholinesterase enzyme activity and using these tools.

In people, who are poisoned due to contact with organophosphorus or carbamate compounds, different signs and symptoms due to degree of toxicity were observed. In weak toxicity, symptoms like tiredness, weakness, vertigo, nausea and dimness were seen, in moderate toxicities, headache, sweating, tearing, saliva increasing, vomiting and limited vision were seen and in severe toxicities, abdominal ache, polyuria, diarrhea, muscle trembling, tottering, pupil stricture, hypotension bradycardia, breathing disorders and probably dead if they didn't be curved on time were seen (Parron *et al*., 1996). Researchers have showed that workers who work only with carbamate toxins (group 2) have more various symptoms than those who work with phosphorus toxins (group 1), this differences depends on the kind of the pesticide, amount of the insecticide which used, way of the toxin absorption and the time in which they contact with toxins. Because in this study spray workers had no notice to kinds of the insecticides and its formulations and these two factor have important affects on occurring the toxicity (decrease of cholinesterase enzyme activity), or its symptoms, so more studies in this subject are needed and also because of the done studies in Iran are very limited. For example studies have showed that slow releasing formulation of 5% malathion was safe and caused no symptoms of the toxicity (Lal *et al*., 2004). The malathion insecticide have less preventing effect on cholinesterase activity than diazinon and caused less side effects and was more safe.

In this study no significant relationship was seen between the blood cholinesterase activity in spray workers and age groups, history of the spray workers, knowledge, cigarette smoking, history of toxicity and responsibility of them ($p>0.05$). Other studies showed that there was no significant relationship between responsibility of workers and decrease of cholinesterase activity after toxicity with organophosphorus toxins (More *et al*., 2003) which confirmed present study. But there was significant relationship between using protective personnel tools and having clinical symptoms and cholinesterase reduction activity ($p = 0.04$, $p = 0.02$ and $p<0.05$, respectively).

According to this point that studies were done by researchers in Iran are very limited, further studies about the assessment of different effects of various kinds of organophosphorus pesticides and relationships to the factors like time of contacts, time of poisoning, environment temperature and observing the hygienic points should be conducted. The measurement of pre-exposure cholinesterase activity level is essential for comparison of the values after pesticide application.

In conclusion, the analysis of the data demonstrated that no significant relationship between spray worker blood cholinesterase activity and age groups, history of the work, knowledge, cigarette smoking, history of the toxicity and their responsibilities were observed. Education of the spray workers and the all persons who have contact with the toxin compounds and pesticides is necessary.

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