Research Article

Assessment of Anticholinesterase Toxicity, Oxidative Stress and Antioxidant Status in Carbamate and Organophosphorus Pesticides-Exposed Agricultural Workers

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ABSTRACT

Among the numerous pesticides, anticholinesterase compounds are widely used. Their toxicity induced by cholinesterase inhibition at the synapses and neuromuscular junctions, leading to neurological disorders. Inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) has been used as sensitive biomarkers for pesticides exposure. In the present study, AChE and BuChE levels were estimated in agricultural workers exposed to carbamate and organophosphorus pesticides with average 9.8±3.5 years relative to the controls. The toxic effects of pesticides may be attributed to induction of oxidative stress and alteration in antioxidant system. Our results showed significant decrease in AChE and BuChE levels with inhibition percentage of 39% and 61% respectively, in exposed workers than controls. Additionally, there was a significant increase in malondialdehyde (MDA) as an oxidative stress marker. Concerning antioxidant status, there was significant decrease in reduced glutathione (GSH) levels while there were significant increases in activity of glutathione dependent enzymes, glutathione peroxidase (GPx) and glutathione-S-transferase (GST). On the other hands, there were significant decreases in enzymatic antioxidants, super oxide dismutase (SOD) and catalase. A negative correlation was found between BuChE activity and MDA levels. So, it was concluded that evaluation of BuChE inhibition may be sensitive tool for assessing the risk of oxidative stress induced after occupational pesticides exposure.

Keywords: pesticides, carbamate, organophosphorus AChE, BuChE, antioxidant enzymes, oxidative stress.

INTRODUCTION

Pesticides are extensively used chemicals all over the world in many occupational and residential fields to control pests and prevent pests-induced diseases[1]. Organophosphorus (OP) and carbamate compounds are the most commonly used pesticide in agriculture field[2]. Their toxicity depends largely on their ability to inhibit cholinesterase activity[3]. There are strong associations between symptoms of pesticides toxicity and reduction of cholinesterase activity in exposed populations[4],[5]. For this reason, measurement of blood AChE and BuChE activity have been widely used as a biomarker for cholinesterase-inhibiting pesticides[6]. The cholinesterase that found in neural tissues and the erythrocyte membrane is known as serum or true acetylcholinesterase (AChE)[7]. On the other hands, plasma cholinesterase (BuChE) or pseudo-cholinesterase is synthesized primarily in the liver and released at a high rate into plasma[8]. Cholinesterases catalyze the hydrolysis of the neurotransmitter such as acetylcholine (ACh) into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation[9]. Carbamates and OP pesticides inhibit cholinesterases enzymes either by carbamylating or phosphorylating its active site. The enzymatic inhibition prevents acetylcholine hydrolysis causing accumulation of the ACh neurotransmitter in the synaptic cleft[10]. Depending on the degree of AChE inhibition, the cholinergic overstimulation may result in hyperactivity, causing, seizures, severe muscle paralysis, coma, respiratory failure, and even death[11].

In most cases, the metabolism of pesticides may result in generation of free radicals and reactive oxygen species (ROS) besides releasing of highly reactive metabolites[12]. By this way, pesticide exposure may disturb the oxidative stress homeostasis of the cell by altering antioxidant defense mechanisms[13]. The produced ROS can interact with main macromolecules of the cell causing damage to nucleic acids, proteins, lipids, and carbohydrates. Malondialdehyde (MDA) is considered as a product of lipid peroxidation and widely used by many researchers as a biomarker for pesticides-induced oxidative stress. If the body couldn't overcome the increasing rate of induced oxidative stress, it would promote carcinogenic mutations via induction of oxidative DNA damage[14]. To prevent oxidative damage of pesticide, defensive systems of enzymatic and non-enzymatic antioxidants scavenge the free radical and ROS. The antioxidant enzymes include glutathione peroxidase (GPx), glutathione-S-transeferase

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(GST), catalase, superoxide dismutase (SOD) and non-enzymatic antioxidant reduced glutathione (GSH)[15]. Excessive consumption of GSH and change in different antioxidant activity represents a biomarker for the toxicity caused by pro-oxidant xenobiotics such as carbamates and OP pesticides[16]. Therefore, the present study was aimed to evaluate the alteration in AChE and BuChE activities as sensitive biomarkers of pesticides exposure among agriculture workers. Additionally, we aimed to evaluate oxidative stress, enzymatic and non-enzymatic antioxidant status in pesticide-exposed agricultural workers.

METHODOLOGY
Study population
The present study was conducted on 51 workers occupationally exposed to pesticides for more than 5 years, and 50 non-occupationally exposed controls. The two groups were recruited from village in El Monofya governorate, Egypt and matched in their socioeconomic status, age, and smoking habits. Approval of the ethics committee was obtained from the Ethical Committee in the National Research Centre, Egypt. A written consent was taken from all the selected subjects. The workers were exposed to mixture of pesticides mainly carbamate and organophosphorus with a mean duration (9.8±3.5) years. Considering the low socioeconomic status of pesticides-exposed workers, they mostly handle pesticides with their hands using little or no protective equipment that aren't effective to protect them for pesticides while mixing, loading or spraying stages.
Sample collection
About 6 ml of the venous blood from each subject was collected and divided into three separate tubes. Three ml blood in dry tube was left for 30 min at room temperature to coagulate then centrifuged for 10 minutes at 3000 rpm for separating serum for AChE and MDA determination. Another two ml blood was collected in an EDTA tube, firstly to estimate GSH in whole blood then centrifuged for 3000 rpm for 10 minutes to separate plasma for determination of BuChE, catalase and GST activities. Finally, one ml of EDTA blood was centrifuged at 40000 rpm for 10 min for separating red blood cell which was washed with normal cold saline for determination of SOD and GPx activities. All samples were stored at -70º C until determination by colorimetric method.
Biomarkers of exposure
Acetylcholinesterase AChE level
An immunoassay kit was used for in vitro quantitative determination of human acetylcholinesterase concentration in serum by (Sandwich-ELISA, Sunredbio, Shanghai).
BuChE activity
The activity of butyrylcholinesterase was measured in plasma by colorimetric method according to Knedel and Bottger [17].
Biomarker of oxidative stress
MDA concentration
Serum MDA activity was determined in serum colorimetrically[18].

Antioxidant biomarkers
Non-enzymatic:
GSH concentration
Glutathione reduced (GSH) concentration was determined in whole blood sample by colorimetric method[19].
Enzymatic:
Catalase activity
Plasma catalase activity was estimated in plasma using end point colorimerty[20].
SOD activity
Superoxide dismutase (SOD) activity was determined calorimetrically [21].
GST activity
Glutathione-S-Transeferase (GST) activity was determined in plasma by colorimetric method[22].
GPx activity
Glutathione peroxidase (GPx) was determined using colorimetric method[23].
Statistical analysis
Statistical analysis was done through SPSS version 18. Quantitative results were expressed as mean and standard deviation (Mean ±SD). The comparisons between two groups were done through independent t-test and independent Mann- Whitney- u test. The qualitative results were analyzed using Person's Chi-square (χ^2). Correlation coefficient was used to study the relationship between two quantitative variables. P-value <0.05 was considered significant. All the results were tabulated and the suitable figures were illustrated.

RESULTS
Fifty one pesticides-exposed agricultural workers and 50 unexposed controls were included in our study. There was no significant difference in age (P=0.781) between workers and control groups (35.4±11.8 and 33.4±7.5 respectively). About 18 (35%) of exposed workers were smokers while 33 (65%) were not. In contrast, 16 (32%) of control subjects were smokers while 34 (68%) were not smokers. There was no significant difference in smoking habit (P=0.892) between the two groups. The agricultural workers of the present study were occupationally exposed to various types of pesticides for about 141.5±80.6 day/year (Table1).
Table 2 showed a significant decrease in AChE level and BuChE activity in the pesticides-exposed workers compared to the unexposed control group with percentage of inhibition of 39% and 61% respectively. It was also revealed a significant decrease in SOD activity, GSH concentration and catalase activity level in the workers than control subjects. On the other hand, the MDA concentration, GPx and GST activities were significantly increased in workers compared to controls.
Table (3) shows Pearson's correlation analysis between AChE and BuChE with MDA and antioxidant biomarkers exposed workers. There was significant positive correlation between AChE and BuChE inhibition. The reduction in AChE was significantly correlated with the decrease in activity of catalase while BuChE was positively correlated with both GSH level and catalase

Table 1: List of common pesticides used by agricultural workers in the study area.

Common name	Pesticide use	Chemical classification	CAS no.	WHO
Malathion	Insecticide	Organophosphorus	121-75-5	III
Dimethoate	Insecticide	Organophosphorus	60-51-5	II
Chloropyrifos	Insecticide	Organophosphorus	2921-88-2	II
Carbofuran	Insecticide	Carbamate	1563-66-2	Ib
Glophosate	Herbicide	Organogosphorous	1071-83-6	III
Mancozeb	Fungicide	Dithiocarbamate	8018-01-7	U

CAS no.: CAS Registry number, Ib: highly hazardous, II: moderately hazardous III: slightly hazardous, U: unlikely to present acute hazard in normal use[24].

Table 2: The difference in the measured biomarkers between pesticides-exposed workers and control subjects.

Group Statistics	Control subjects (50) Mean±SD	Exposed workers (51) Mean±SD
Toxicity markers		
AChE(ng/ml)	556.9±374.6	327.4±217.6[*a]
BuChE (U/L)	3700.2±1050.9	2616.7±561.1[a]
Oxidative stress marker		
MDA(nmol/ml)	2.344±8.823	6.51±1.95[b]
Antioxidant status		
GSH(mg/dL)	40.68±12.763	22.40±6.04[b]
GST (U/L)	321.67±116.24	446.97±179.46[b]
GPx(mU/Ml)	294.25±174.89	526.65±260.09[*b]
SOD (U/ml)	197.40±75.27	153.94±55.22[b]
Catalase(U/L)	558.96±105.53	499.25±113.89[c]

Independent Mann- Whitney- u test *
a data was significant at P<0.001
b data was significant at P<0.0001
C data was significant at p<0.005

Table 3: correlations of the cholinesterase levels with oxidative stress and antioxidant biomarkers.

	AChE	BuChE
AChE	-------	
BuChE	0.3*	-------
MDA	-0.03	-0.52*
GSH	0.81	0.33*
GST	-0.11	-0.11
GPx	-0.19	-0.21
SOD	0.14	0.34
Catalase	0.31*	0.22*

* Correlation is significant at the 0.05

activities and negatively correlated with MDA level. Additionally, the duration of exposure was significantly negatively correlated with decline in BuChE activity (fig 1) and non-significantly related inversely to AChE.

DISCUSSION

The inhibition in AChE and BuChE activities is commonly used as biomarkers of the occupational and environmental exposure to both OP and carbamates pesticides[5]. The advantages of these biomarkers for prediction of pesticides exposure may be because of their high sensitivity, easy measurement and depending on the level of exposure[25].The results of the present study showed a significant decrease in AChE and BuChE levels in workers occupationally exposed to pesticides in comparison to the unexposed control group. These results are consistent with the previous studies [26, 27]. Moreover, the results of this study revealed that the percentage of BuChE inhibition was higher than AChE (61%, 39% respectively). Dhananjayan and his team observed a significant reduction in BuChE enzyme activities than AChE among children's environmentally exposed to pesticides[28]. According to Ellison et al., the inhibition of BuChE was more than AChE in a group of Egyptian farmers exposed to different pesticides[29]. The authors concluded that BuChE appeared to be more sensitive biomarker[29]. The BuChE can be considered as an endogenous scavenger of anticholinesterase compounds such as carbamates and organophosphorus pesticides[9]. The greater inhibition in BuChE activities may be due to its role as a detoxifying enzyme that could hydrolyze hydrophobic and hydrophilic carboxylic or phosphoric acid ester containing compounds[9]. In the present study, a negative correlation between the inhibition in BuChE activities and the period of exposure to pesticides (day/year) in exposed workers was observed. This correlation may reflect the high sensitivity of BuChE as a biomarker for inhibiting-cholinesterase pesticides.

In addition to the inhibitory effect of pesticides exposure on cholinesterase activity, pesticides act as powerful promoter of many cellular pathways that are associated with oxidative stress. Pesticides-induced oxidative stress can be either in the form of overproduction of free radicals or alteration in antioxidant defense mechanisms, including detoxification or scavenging enzymes[30]. Pesticides induced oxidative stress can be confirmed by the direct measurement of lipid peroxidation by-product malondialdehyde (MDA)[15]. There is increasing evidence that pesticides induced oxidative stress through the generation of oxygen free radicals, leading to lipid peroxidation and increased MDA level[30]. The results of the current work showed an increased MDA levels in the pesticide-exposed workers compared to the control group. This result was Consistent with a previous study that reported an increased MDA levels in farm workers and pesticides applicators than the controls[15]. In addition, the present study found an inverse correlation between BuChE and MDA activities. The increased free radical production may lead to H_2O_2 mediated oxidation of amino acids at the active site of the cholinesterase enzyme that causes enzymatic inhibition[31].
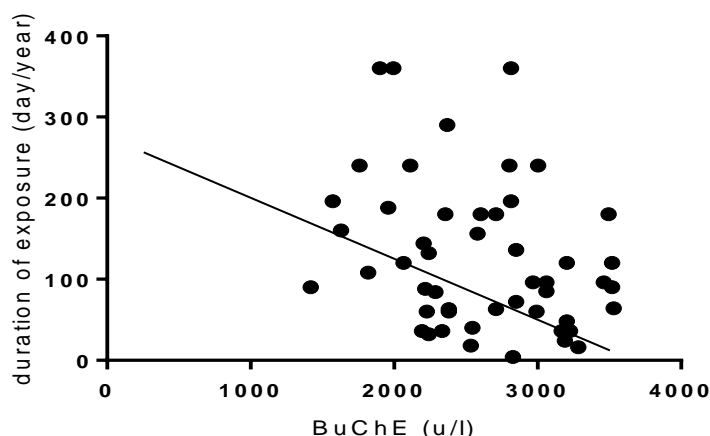
The antioxidant enzymes including SOD and catalase

Figure 1: the positive correlation between decrease in BuChE activity and duration of exposure (day/ year). r= 0.31, P<0.05 level (2-tailed).

represent the first line of defense against risk of oxidative stress. SOD protects the body against highly reactive superoxide anions (O^{2-}) by converting them into hydrogen peroxide (H_2O_2). Catalase is responsible for the catalytic decomposition of H_2O_2 to O_2 and H_2O[32]. Our results showed a significant decrease in both SOD and catalase activities in exposed workers compared to control subjects. These results are in agreement with previous studies of Prakasam et al. and Lŏpez et al.[32, 33]. Decreased catalase activities in response to pesticide exposure might reduce the body protection against toxic effect of free radicals. In long-lasting exposure to pesticides, free radicals and reactive oxygen species simply consume and exhaust antioxidant agent present in the body[30]. The generated oxygen radicals might inhibit thiol groups of catalase and result in the accumulation of excess hydrogen peroxide that may further inhibit SOD[34]. This suggestion is consistent with the lower catalase and SOD activities observed in the current study.

The present work revealed that the concentration of reduced glutathione (GSH), which is a key cellular non-enzymatic antioxidant, was significantly decreased in pesticides exposed workers than unexposed control. It directly participates in the scavenging of free radicals, hydrogen peroxide, superoxide anion and hydroxyl radicals[32]. The reduction in GSH level may be attributed to its role as a cofactor for GPx enzyme that reduces H_2O_2 and other peroxidases. As a result of this reaction, GSH is oxidized and therefore the reduced glutathione level is diminished[35]. According to these results, a significant increase in both GPx and GST activities in the exposed workers relative to the controls was observed. The GST function is to detoxify toxic compounds by binding them to inactivate electrophilic compounds utilizing GSH as in its detoxification reaction. It was thought that the reduction in glutathione was associated with the increase in GST and GPx activity[36]. This could be another explanation of the reduction in GSH levels in the exposed workers than controls. The decrease in GSH availability could have considerable influence on the metabolism and rate of detoxification of pesticides.

In conclusion, the change in BuChE and MDA activities can be used as an indicative tool for detecting the extent of pesticide toxicity among exposed workers. Antioxidants levels could be an indicative of the endogenous protective status against pesticides chronic toxicity. It is recommended that the worker's cholinesterase levels and oxidative stress biomarker should be periodically evaluated for workers exposed to pesticides. Additionally, antioxidant supplementations could be of great value in prevention of chronic hazardous effects of pesticides exposure, further studies are recommended.

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