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## Electrometric Determination of Blood Cholinesterase Activities in Workers Exposed to Insecticides in Mosul, Iraq

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**Abstract:** The purpose of the present study was to apply a modified electrometric cholinesterase method for measurement of plasma and erythrocyte cholinesterase activities in subjects exposed to insecticides during their routine work. The subjects (n = 40) of both sexes were farmers (31) and veterinarians (9) from Mosul, Iraq and none was suffering from any overt health problems. A modified electrometric method was used to measure plasma and erythrocyte cholinesterase activities using 0.2 mL blood sample, 0.1 mL of 7.1% acetylcholine iodide as a substrate with incubation at 37°C for 20 min. Cholinesterase activities of the subjects were compared with reference values determined simultaneously. The percentage of plasma cholinesterase inhibition in the subjects (n = 23) ranged between 11-70% that comprised 10-20% inhibition in 14 subjects, 21-40% in 6 subjects and 41 to 70% in 3 subjects. Erythrocyte cholinesterase inhibition in the subjects (n = 18) ranged between 11-51% comprising 10-20% inhibition in 13 subjects, 21-40% in 4 subjects and 51% in one subject. The data suggest the value of the modified electrometric method for monitoring blood cholinesterase inhibition in workers exposed to insecticides and further stress the need for frequent blood cholinesterase monitoring programs to include a wider range of workers in Iraq occupationally exposed to anticholinesterase pesticides.

**Key words:** Organophosphate, carbamate, cholinesterase, biomonitoring, pesticide exposure

### INTRODUCTION

Domestic use of organophosphate and carbamate insecticides in Iraq is frequent and widespread and presents a serious public health concern. These insecticides act by inhibiting cholinesterase (ChE) activity in the nervous tissues and neuromuscular junctions causing accumulation of acetylcholine at the nerve endings and producing parasympathetic hyperstimulation manifested by nicotinic, muscarinic and central nervous system effects (Wilson, 1998; Kwong, 2002; Rusyniak and Nanagas, 2004). Occupational exposures to pesticides are mostly through inhalation, dermal and ocular (Singh *et al.*, 2002; Muttray *et al.*, 2006). Practicing veterinarians can be exposed to pesticides through applying insecticides for pest control in animals (Coggon, 2002; Jaga and Dharmani, 2003).

Measurement of blood (plasma or erythrocyte) ChE activities is a biomarker for monitoring exposure to organophosphate and carbamate insecticides and used to diagnose poisoning induced by these insecticides (Wilson *et al.*, 1998, 2005; Wilson, 1999). Usually a 20-30% decrease in blood ChE activity indicates exposure to ChE inhibitors (Lotti, 1995; Wilson *et al.*, 2005) and further enzyme inhibition occurs in cases of pesticide-related serious illnesses (Jaga and Dharmani, 2003). Various colorimetric and electrometric methods exist for measurement of ChE activities in the blood and other tissues such as the brain (Fairbrother *et al.*, 1991; Wilson, 1999). One of the common methods for measuring blood ChE activities is the electrometric method of Michel (1949) which is based on the

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hydrolysis of acetylcholine and production of acetic acid which subsequently decreases the pH of the reaction medium (Wills, 1972; Wilson, 1999). The method has undergone various modifications to shorten the assay procedure and to increase its throughput (Wills, 1972; Wilson, 1999). A recent modification of the electrometric method has been described for determination of blood ChE activities in man (Ahmed and Mohammad, 2005; Mohammad *et al.*, 2006a) and animals (Mohammad *et al.*, 1997, 2005, 2006b).

An important aspect of health care for veterinarians and agricultural workers is the surveillance of pesticide-related illness associated with exposure to organophosphate or carbamate insecticides. Surveillance for assessing exposure of workers to anticholinesterase insecticides includes measuring ChE activities in erythrocytes and plasma or serum (Wilson *et al.*, 1998, 2005; Coggon, 2002; Jaga and Dharmani, 2003; Mutray *et al.*, 2006). The purpose of the present study was to apply a modified electrometric method for measurement of plasma and erythrocyte ChE activities in veterinarians and farmers exposed to insecticides during their routine work in Mosul, Iraq.

## MATERIALS AND METHODS

### Subjects

The subjects (n = 40) of both sexes were farmers (31) and veterinarians (9) from Mosul, Iraq and none of them was suffering from overt health problems. They were exposed to various insecticides for at least one year during their routine work with insecticide handling and application for pest control. The types of insecticides and frequency of exposure to insecticides and/or other chemicals could not be verified from these volunteers. Informed consents were obtained from the volunteers before the start of the study.

### Electrometric Method for Measurement of Plasma or Erythrocyte ChE Activities

Venous blood samples were collected from human volunteers using heparinized test tubes. Plasma was separated from erythrocytes by centrifugation at 3000 rpm (Centurion, U.K.) for 15 min. The modified electrometric method was used for measurement of plasma and erythrocyte ChE activities (Mohammad *et al.*, 1997; Ahmed and Mohammad, 2005). The enzymatic reaction mixture contained 3 mL distilled water, 0.2 mL plasma or erythrocytes and 3 mL, pH 8.1 barbital-phosphate buffer (Mohammad *et al.*, 1997; Ahmed and Mohammad, 2005). The pH of the mixture (pH1) was measured with a glass electrode using a pH meter (Consort, Belgium), followed by the addition of 0.1 mL of aqueous solution of acetylthiocholine iodide (7.5%). The reaction mixture was incubated at 37°C for 20 min. At the end of the incubation period, the pH of the reaction mixture (pH2) was measured again.

The enzyme activity was calculated as follows:

$$\text{ChE activity } (\Delta\text{pH}/20 \text{ min}) = (\text{pH1}-\text{pH2})-\Delta \text{ pH of blank}$$

The blank was without plasma or erythrocytes. Cholinesterases of pesticide-exposed subjects were compared with reference (control) values of healthy subjects determined simultaneously. The latter reference values were reported elsewhere (Ahmed and Mohammad, 2005). The percentage of enzyme inhibition was calculated as follows:

$$\% \text{ inhibition} = [(\text{ChE activity of unexposed}-\text{ChE activity of exposed})/\text{ChE activity of unexposed}] \times 100$$

## RESULTS

Table 1 shows the ranges of plasma and erythrocyte ChE activities and the percentages of their inhibition in subjects exposed to various insecticides. The percentage of plasma ChE inhibition in the

Table 1: Plasma and erythrocyte cholinesterase (ChE) activities ( $\Delta$ pH/20 min) and their inhibition in workers exposed to insecticides

| Range of ChE activity ( $\Delta$ pH/20 min) | Male            |   |                | Female          |   |                |
|---|-----------------|---|----------------|-----------------|---|----------------|
|   | No. of subjects | Mean ChE activity ( $\Delta$ pH/20 min) | Inhibition (%) | No. of subjects | Mean ChE activity ( $\Delta$ pH/20 min) | Inhibition (%) |
| <b>Plasma</b>                               |                 |   |                |                 |   |                |
| < 0.60                                      | 1 (F)           | 0.32                                    | 70             | 2 (V)           | 0.48                                    | 47             |
| 0.60-0.80                                   | 6 (F and V)     | 0.70                                    | 33             | 1 (V)           | 0.74                                    | 19             |
| 0.81-1.00                                   | 13 (F)          | 0.93                                    | 11             | none            |   |                |
| 1.10-1.15                                   | 16 (F and V)    | 1.20                                    | 0              | 1 (V)           | 1.11                                    | 0              |
| <b>Erythrocyte</b>                          |                 |   |                |                 |   |                |
| < 0.70                                      | 1 (F)           | 0.58                                    | 51             | none            |   |                |
| 0.70-0.90                                   | 4 (F)           | 0.80                                    | 32             | none            |   |                |
| 0.91-1.00                                   | 13 (F and V)    | 1.05                                    | 11             | none            |   |                |
| 1.10-1.53                                   | 18 (F and V)    | 1.20                                    | 0              | 3 (V)           | 1.27                                    | 0              |

F = Farmer, V = Veterinarian. Reference plasma ChE activity ( $\Delta$ pH/20 min): Male = 1.05, Female = 0.91; Reference erythrocyte ChE activity ( $\Delta$ pH/20 min): Male = 1.18, Female = 1.19 (Ahmed and Mohammad, 2005)

Table 2: Mean values of plasma and erythrocyte cholinesterase (ChE) activities inhibited in workers exposed to insecticides

| Variable                           | Male | Female |
|------------------------------------|------|--------|
| <b>Plasma</b>                      |      |        |
| n                                  | 20   | 3      |
| ChE activity ( $\Delta$ pH/20 min) | 0.83 | 0.57   |
| % inhibition                       | 21   | 37     |
| <b>Erythrocyte</b>                 |      |        |
| n                                  | 18   | none   |
| ChE activity ( $\Delta$ pH/20 min) | 0.79 |        |
| % inhibition                       | 33   |        |

Reference plasma ChE activity ( $\Delta$ pH/20 min): Male = 1.05, Female = 0.91; Reference erythrocyte ChE activity ( $\Delta$ pH/20 min): Male = 1.18, Female = 1.19 (Ahmed and Mohammad, 2005)

subjects (n = 23) ranged between 11-70% that comprised 10-20% inhibition in 14 subjects, 21-40% in 6 subjects and 41 to 70% in 3 subjects. Erythrocyte ChE inhibition in the subjects (n = 18) ranged between 11-51%, comprising 10-20% inhibition in 13 subjects, 21-40% in 4 subjects and 51% in one subject. Overall percentages of plasma ChE inhibitions in males and females (compared to reference values) were 21 and 37%, respectively (Table 2). The percentage of erythrocyte ChE inhibition in male workers (compared to the reference value) was 33%; female erythrocyte ChE activity (n = 3) was not significantly affected (Table 2).

## DISCUSSION

Biological monitoring of occupational exposure to anticholinesterase pesticides (organophosphates and carbamates) includes a method of surveillance by measuring blood ChE activities (Wilson, 1998; Coggon, 2002; Jaga and Dharmani, 2003; Wilson *et al.*, 2005). The findings of the present study suggest exposure of the farmers and veterinarians to anticholinesterase insecticides as indicated by their depressed ChE activities in the blood. We assume that the type of exposure was gradual and in small doses, since the subjects did not complain from any overt anticholinesterase symptoms or adverse health problems. However, further health care are needed for these workers as subtle adverse effects cannot be detected immediately during exposure and some effects might appear several months later (Coggon, 2002; Jaga and Dharmani, 2003; He, 2002). Handling and/or exposure to such insecticides due to occupations of the subjects could often result in inhibition of blood (erythrocyte and/or plasma) ChE activities (Singh *et al.*, 2002; Jaga and Dharmani, 2003; Muttray *et al.*, 2006). However, ChE activity can also be measured to assess acute organophosphate or carbamate toxicity from any exposure, including the nonoccupational situations, since ChE depression is diagnostic of toxicosis

induced by these pesticides (Lotti, 1995; Wilson, 1998; Kwong, 2002; Rusyniak and Nanagas, 2004). People with chronic exposure to anticholinesterase insecticides at low to moderately high doses can develop a pesticide-related neurological illness (Coggon, 2002; He, 2002; Jaga and Dharmani, 2003).

The two important enzymes measured for biological monitoring are erythrocyte ChE (EC 3.1.1.7 under the enzyme nomenclature system of the International Union of Biochemistry and Molecular Biology) and serum (plasma) or pseudo ChE (EC 3.1.1.8) (Wilson, 1998, 1999). It is recommended that both enzymes should be measured; the findings of both measurements could be significant and useful in assessing pesticide-induced toxicosis (Singh *et al.*, 2002; Jaga and Dharmani, 2003; Wilson *et al.*, 2005; Muttray *et al.*, 2006). Marginal ChE inhibition is difficult to assess unless pre-exposure ChE values have been determined in advance for each subject (Lotti, 1995; Jaga and Dharmani, 2003; Rusyniak and Nanagas, 2004; Wilson *et al.*, 2005). Enzyme inhibition should be at least 15% to be considered significant and 20-30% ChE inhibition is considered an indication of exposure (Lotti, 1995; Wilson, 1999; Jaga and Dharmani, 2003). In the present study, mean inhibitions of plasma and erythrocyte ChE activities were between 21 to 37% with some individuals having ChE inhibition by >50% (Table 1). The risks of pesticides exposure are highest for agricultural workers and veterinarians (Das *et al.*, 2001; Jaga and Dharmani, 2003; Muttray *et al.*, 2006) and they should be well educated on pesticides toxicity for preventing exposure that might result in adverse effects.

It appears from the findings of the present study and others from our laboratory (Mohammad *et al.*, 1997, 2006a, b; Ahmed and Mohammad, 2005) that the modified electrometric method could have practical applications in detecting ChE inhibition following exposure to insecticides. This method has a short one step-incubation time and it is sensitive enough, inexpensive and simple (Mohammad *et al.*, 1997, 2005, 2006a, b; Ahmed and Mohammad, 2005). In conclusion, the data suggest the value of the modified electrometric method for monitoring blood ChE inhibition in workers exposed to insecticides and further stress the need for frequent blood ChE monitoring programs to include a wider range of workers in Iraq occupationally exposed to anticholinesterase pesticides.

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