

EFFECTS OF OCCUPATIONAL EXPOSURE IN PESTICIDE PLANT ON WORKERS' SERUM AND ERYTHROCYTE CHOLINESTERASE ACTIVITY

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Abstract

Objectives: The determination of cholinesterase activity has been commonly applied in the biomonitoring of exposure to organophosphates and carbamates and in the diagnosis of poisoning with anticholinesterase compounds. One of the groups who are at risk of pesticide intoxication are the workers engaged in the production of these chemicals. **Aims:** The aim of this study was to assess the effect of pesticides on erythrocyte and serum cholinesterase activity in workers occupationally exposed to these chemicals. **Methods:** The subjects were 63 workers at a pesticide plant. Blood samples were collected before they were employed (phase I) and after 3 months of working in the plant (phase II). Cholinesterase level in erythrocytes (EChE) was determined using the modified Ellman method, and serum cholinesterase (SChE) by butyrylthiocholine substrate assay. **Results:** The mean EChE levels were 48 ± 11 IU/g Hb in phase I and 37 ± 17 IU/g Hb in phase II (paired t-test, mean = -29; 95% CI = -43–14, $p < 0.001$). The mean SChE level was 9569 ± 2496 IU/l in phase I, and 7970 ± 2067 IU/l in phase II (paired t-test, mean = 1599; 95% CI = 1140–2058, $p < 0.001$). There was a significant increase in ALT level ($p < 0.001$) and a decrease in serum albumin level ($p < 0.001$). **Conclusion:** In view of the significant decrease in EChE and SChE levels among pesticide workers, it seems that routine assessment of cholinesterase level in workers employed in such occupations and people handling pesticides should be made obligatory.

Key words:

Pesticide, Organophosphate, Cholinesterase, Occupational

INTRODUCTION

Cholinesterase is one of the most important enzymes; it is required for the proper function of the nervous system. There are two cholinesterase enzymes in humans which are capable of hydrolyzing acetylcholine: (1) acetylcholinesterase (EC 3.1.1.7), the true cholinesterase or cholinesterase I, which is present in the erythrocytes, nerve ending, lung and spleen, and (2) acetyl cholinesterase hydrolyzes (EC 3.1.1.8), pseudocholinesterase or cholinesterase II, which is found in the serum, pancreas, and liver [1,2].

Some of the chemical classes of pesticides such as organophosphates and carbamates either interfere with or inhibit

the activity of these enzymes [3]. Annually, about 3 million people worldwide are intoxicated with organophosphates; out of this amount, 300,000 either die or are severely injured [4]. Although the signs of cholinesterase inhibition by carbamates are similar to those induced by organophosphates, in carbamate intoxication, blood cholinesterase level faster regains its normal value. This period of time, depending on pesticide dose, varies from a few hours to a few days for carbamates and from a few days to a few weeks for organophosphates [5]. The erythrocyte cholinesterase (EChE) and serum cholinesterase (SChE) return to normal after 82 days and 50 days, respectively, if the intoxicated person is no

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longer exposed to pesticides [6]. Cholinesterase activity decreases by 15–25%, 25–35%, and 35–50% in low, moderate and severe intoxication with pesticides, respectively.

EChE determination indicates whether the person has been under long-term or chronic pesticide exposure, whereas SChE activity is a good indicator of acute poisoning with organophosphates. It is recommended that the worker's cholinesterase level should be assessed before he starts working at a pesticide plant. As the concentration range considered to be the baseline value for an individual is very wide (4000–11 000 IU/l), the variation in cholinesterase level may be significant in one person but still remain within the normal range [7]. The aim of this study was to evaluate the health effects of occupational exposure of pesticide plant workers and the influence of this exposure on the EChE and SChE activities.

MATERIALS AND METHODS

The present project was a cohort study of workers at a pesticide plant in Iran that was conducted in 2005. Before the beginning of employment (phase I), every worker who agreed to take part in this study completed a questionnaire and had a 5 ml blood sample collected. An amount of 2 ml blood was placed into a test tube containing anticoagulant (EDTA) for EChE level determination, and 3 ml blood was used for serum preparation to determine serum albumin, aspartate transaminase (ALT), alanine transaminase (AST) and SChE levels. The study procedure was repeated after 3 months (phase II). In total, 63 workers participated in the two phases of the experiment lasting for a period of three months. Blood samples were transported to a laboratory and after centrifugation the sera were separated and stored at -70°C, the remaining blood samples were washed with normal saline, and the compacted erythrocytes were kept at -70°C. Serum albumin was determined using bromocresol green (BCG), while AST and ALT were assayed by the method developed by the International Federation of Clinical Chemistry (IFCC) using Parsazmoon company kit (Iran) and photometer (clinic II, Iran).

For EChE determination, hemoglobin (Hb) in erythrocytes was determined at first, using Drabkin's reagent. Then,

50 μ l blood was added to 3ml distilled water and the tubes were vortexed for 30 seconds. EChE was determined with thiocholine propionyl substrate using the modified Ellman method [8]. The cholinesterase activity was calculated as ChE level per g hemoglobin. SChE test was carried out using Parsazmoon kit with butyrylthiocholine substrate.

The workers were classified into five groups by EChE level variation. The workers for whom EChE level did not show any decrease or increase belonged to group 1. Groups 2 to 5, were the workers for whom cholinesterase activity decreased by < 15%, 15–25%, 26–35%, and more than 35%, respectively. The study population was also classified by workplace into two groups: 34 workers who were not directly involved in pesticide production (group 1), including service, technical, administration, and storage and package workers, and 29 workers directly exposed to pesticides (in powder, liquid fertilizers, herbicides, and insecticides departments) (group 2). 95% confidence interval was adopted for all the statistical analyses. Analysis of variance (ANOVA) and chi-square tests were used for between-group comparisons of continuous and categorical variables. Paired t-test was used for comparing the means of the study parameters in the two phases of the project. The SPSS package was used for statistical analysis.

RESULTS

The subjects' age ranged between 21–53 years (mean 32 ± 8 years). EChE concentration was found to vary from 23–72 IU/g Hb in phase I, with the mean EChE level of 48 ± 11 IU/g Hb. In phase II, EChE concentration ranged from 9–88 IU/g Hb, and the mean EChE level was 37 ± 17 IU/g Hb. The comparison between the two phases showed a decrease in EChE level (paired t-test, mean = -29; 95% CI = -43–14, $p < 0.001$). As for the SChE level, it ranged from 4960–15 420 IU/l, with the mean value of 9569 ± 2496 IU/l. In phase II of the study, SChE concentration ranged from 3700–13 020 IU/l, with the mean level of 7970 ± 2067 IU/l (paired t-test, mean = 1599; 95% CI = 1140–2058, $p < 0.001$).

The mean AST activity was 22 ± 8 IU/l in phase I and 24 ± 10 IU/l in phase II, but the difference was of no

statistical significance. The mean ALT levels in phase I and II were 16 ± 8 IU/l and 24 ± 13 IU/l, respectively (paired t-test, mean = -7; 95% CI = -10–4, $p < 0.001$). Serum albumin concentration was 4.5 ± 0.04 gr/dl in phase I and 4.0 ± 0.7 gr/dl in phase II (paired t-test, mean = -0.5; 95% CI = 0.2–0.7, $p < 0.001$).

In phase II, the analysis by workplace showed the mean SChE and EChE levels of 8307 ± 2283 IU/l, and 36.8 ± 12.2 IU/g Hb, respectively, in group 1, and 7574 ± 1737 IU/l, 38.0 ± 21.4 IU/g Hb, respectively in group 2. An over 25% decrease in EChE and SChE levels was observed in 8 workers (23.5%) in group 1 and 10 workers (34.5%) in group 2. No significant correlation was found between the workplace and the decreased SChE and EChE concentrations. The period of work ranged between 0–7 years (mean 2.5 ± 1.9 years). The workers were classified by the duration of work in pesticide plant into four groups: 13 workers < 1 year of work, 19 people between 1–3 years, 19 people between 3–5 years, and 12 people > 5 years. No significant relationship was found between the work history and decreased SChE and EChE levels.

The mean body mass index (BMI) in the study population was 25 ± 5 kg/m². BMI did not correlate significantly with the decreased level either of EChE or SChE.

In total, 15 persons showed an over 35% decrease in EChE level and 8 subjects presented an over 35% decrease in their SChE activity. Also, in 18 workers (28%), both the SChE and EChE levels decreased more than 25%.

DISCUSSION

In the present study, about 50% (31) of workers showed EChE level decrease of more than 25%. This finding indicates the relevance of investigating enzyme activity and the possible health effects in workers employed at occupations involving pesticide use. During the study, blood sampling was performed before the commencement of work and three months afterwards. The findings revealed that in some workers, the cholinesterase level was significantly reduced though still remaining within its normal range. On the other hand, every worker was his own reference in the method applied, and the baseline level of this enzyme was determined for each worker in phase I. Therefore, the results of the present research can be more accurate and reliable than those of the case-control studies. The study by Vahdati et al. demonstrated that as much as 70–80% of Iranians have a mutation on one allele of the cholinesterase gene [9]. Also in another study, Housaini et al. have shown that there is a significant difference in ChE activity between the normal Iranian and Irish population (7.8 ± 0.1 vs. 5.2 ± 0.09 , $p < 0.01$) [10].

The present study revealed the importance of population screening for cholinesterase activity and enzyme determination in every worker prior to employment in such occupations as pesticide production. The results are consistent with other reports on the pre- and post-exposure status of the workers. In the study by Lakew et al. who examined 81 persons in Ethiopia before and after they had contact with

Table 1: Decrease in EChE and SChE activity, by workplace

Workplace	No change	Decrease < 15%	Decrease 15–25%	Decrease 25–35%	Decrease > 3%	Total
Group 1*						
EChE	10 (29.4%)	4 (11.8%)	3 (8.8%)	11 (32.4%)	6 (17.6%)	34 (100%)
SChE	11 (32.3%)	9 (26.5%)	8 (23.5%)	4 (11.8%)	2 (5.9%)	34 (100%)
Group 2**						
EChE	10 (34.5%)	4 (13.8%)	1 (3.4%)	5 (17.3%)	9 (31.0%)	29 (100%)
SChE	3 (10.4%)	7 (24.1%)	8 (27.6%)	5 (17.2%)	6 (20.7%)	29 (100%)

* Workers not directly involved in pesticide production.

** Workers directly exposed to pesticides at production department.

pesticides, EChE level was found to be significantly decreased ($p < 0.05$) [11]. The study by Robinson et al. on 17 gardeners in western Canada, who were examined pre- and post-exposure to pesticides, showed a significant, 15% decrease in EChE level ($p \leq 0.05$) [12]. But the results of the case-control studies are sometimes contradictory. In a study on 164 farmers and 83 controls, no significant differences in cholinesterase levels were found between the group of cases and controls [13]. In contrast to these findings, Ciesielski et al., in a study on 202 farmers (cases) and 42 controls in Carolina, found a significant decrease in EChE level (30 IU/g Hb vs. 32 IU/g Hb) among the exposed farmers [14]. These differences can be due to the duration of exposure to pesticides or the type of pesticide used.

The above findings indicate the importance of measuring ChE level in the population and determining the baseline level for every worker, prior to employment at workposts involving pesticide exposure.

A study on Mexican farmers revealed a significant difference between SChE activity before and at the end of the job season (paired t-test = 2.7, $p = 0.01$) [15].

In the study on 44 fruit crop spraying workers, Innes et al. found that 7 out of 44 workers had significantly decreased plasma ChE levels. The BMI of these seven workers was lower than of the other workers in the study group [16]. In the present project, however, no significant correlation was found between SChE level and BMI.

A study performed in Malaysia on farmers working on rice paddy demonstrated a significant decrease in SChE level [17]. In a study performed in California on 542 people who were in charge of supplying the pesticides, 4% of subjects had SChE level 50% lower than the baseline level [18].

In other studies, the cholinesterase level measured in pesticide workers and farmers was compared with the normal range. However, this type of comparison cannot demonstrate the real variation, since the enzyme normal range is very wide, and sometimes the changes can be considerable but the enzyme concentration still remains at its normal level. In a study on 41 people in Ecuador who had contact with insecticides, 88% of subjects showed lower than normal EChE level [19].

In our study, there was no significant difference between the decreased SChE and EChE levels and workplace. However, as shown in table 1, in the severe decrease column, the percentage of workers in group 2 was higher than in group 1 (EChE decrease 31% vs. 17%, and SChE decrease 20% vs. 5%). Thus, the workplace may have had influence on the severe decrease in SChE and EChE levels.

Increased activity of serum AST and ALT and decreased albumin concentration is indicative of the damage to the liver cells due to pesticide exposure. An experimental study demonstrated that Methidathion caused a significant increase in AST, alkaline phosphatase, gamma-glutamyl-transferase, and lactate dehydrogenase levels among the cases, but ALT activity was lower than in the controls [20]. The results of this experiment differed completely from the findings of the present study. Here, although AST activity increased during the three months' period of work, this increase was of no statistical significance, but ALT increase was evident. In a study by Kalender et al. on Wistar rats exposed to Diazinon (organophosphate insecticide), albumin, AST, and ALT levels markedly increased after a period of four weeks [21]. The increased AST and ALT activities indicated a liver damage and a reduced capacity for protein synthesis by liver cells. Considering the importance of the liver function in various metabolic processes, this cellular damage can have serious consequences.

In conclusion, in view of the severe decrease in EChE and SChE activities in more than 28% (18) of the pesticide plant workers in the present study, routine assessment of cholinesterase level among workers employed in such occupations as well as among people handling pesticides e.g. the farmers, seems to be strongly recommended.

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