Cholinesterase Status of Some Ethiopian State Farm Workers Exposed to Organophosphate Pesticides

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Abstract: Cholinesterase Status of Some Ethiopian State Farm Workers Exposed to Organophosphate Pesticides: Kibruyisfa LAKEW, et al. Occupational Safety and Health Division, Region 14 Labour and Social Affairs Bureau—The effects of organophosphate (OP) pesticide exposure on cholinesterase (ChE) activities were assessed in relation to occupational factors by studying 430 pest control workers from three state farms and 161 comparable controls. Plasma cholinesterase (PChE) and erythrocyte cholinesterase (AChE) activities were determined by an electrometric method. Plasma alkaline phosphatase (AP) and glutamic pyruvic transaminase (GPT) were estimated colorimetrically by using diagnostic kits 6391 and 1442520 respectively. Information on OP poisoning symptoms and related occupational factors were collected based on the WHO recommendations. Pest control workers had significantly lower PChE and AChE activities than the controls (P<0.001). In addition, 58 (13.5%) and 47 (10.9%) of the exposed workers had PChE and AChE activities below the lower limits of the controls respectively. Normal plasma AP and GPT values without significant difference from the controls were noted. Among the exposed population, the "spray men" and those who were exposed for 5 years or less were found to be the most affected groups. Although poisoning symptoms were more frequent in the exposed workers than in the controls, only sweating was found to have an association with a significant reduction in AChE activities (P<0.05). The study revealed that the health risks are more apparent in the exposed workers and are of immediate concern. Pre-placement and periodic medical examination and creation of awareness among the exposed population are recommended to minimize the problem.

Key words: Organophosphates, Cholinesterases, Job category, Length of employment, Poisoning symptoms

Organophosphate pesticides are neuroactive molecules which act by disrupting the recovery mechanism of cholinergic neuronal transmission. They inhibit the enzyme ChE which plays an important physiological role in the transmission of nerve impulses to nerve or effector cells in the body. Ethiopia is predominately an agricultural country, and pesticides are extensively used to increase crop yields. The state sector (mainly the Ministry of State Farms Development (MSFD)) uses large quantity of these pesticides. Although pesticides are designed to eliminate undesirable organisms that damage crops, they are potentially dangerous to the nontarget organisms including human beings. Agricultural workers are at particularly high risk for pesticide related illnesses. By job category, these are primarily the mixers and applicators who handle agrichemicals directly. Workers are also exposed to OPs from contact with their residues or treated plant residues.

Cholinesterase inhibition is used as an indirect indicator to monitor OP exposure, but in Ethiopia there is no information about the effects of continuous occupational exposure on ChE levels and on certain enzymes related to liver functions, which may be affected by chronic exposure to OPs. This study has therefore been undertaken to evaluate the health effects of OPs, using ChE and liver function parameters of pesticide exposed state farm workers in relation to OP poisoning symptoms, different job categories and length of employment.

Methods

1. Study sites and study subjects

The study was conducted in three major state farms namely, the Northern Omo, the Wollega and
the Upper Awash Agro-industry Farms. They are about 550 km southwest, 400 km northwest and 200 km east of the capital, Addis Ababa respectively. The average altitude, rainfall and temperature distribution of these state farms differ slightly. Agricultural activities are mainly rain-fed; but the agro-industry farms also use extensive irrigation systems. In all the state farms, OPs were commonly used against notorious pests in cotton, tobacco, maize, fruits and vegetables. Major OP pesticides used in the state farms during the study period are shown in Table 1. This Table shows the toxicity category of pesticides adopted from WHO.

All pest control workers, farm managers and control subjects were informed about the aim of the study and agreed to participate accordingly. Thus all measurements were made with the consent of the subjects. A total population of 591 subjects (all male) participated in this study. The first 430 were pest control workers from the aforementioned state farms. All pesticide workers who participated in this study were permanent employees involved in pest control activities on the state farms. The workers have therefore been exposed to pesticides since their employment as pest control workers. They were continuously exposed to OP pesticides, but they may also have been concurrently exposed to other pesticides where this condition is unavoidable. It was not possible to record the exposure for separate OP pesticides because there was no specific work schedule and pesticides were mostly sprayed in mixtures. The length of employment as pest control workers ranges from 1 month to 30 years. Since all the farms are located in relatively hot areas, pest control operations were commonly conducted from 6:30 to 12:30 in the morning. Each worker is therefore exposed to pesticides for about 6 hr per day, 6 days a week. The study was conducted without affecting the normal pest control activities. Each worker provided a blood sample immediately after completing his work and there was no significant time lapse between the last exposure and the time of blood sampling.

It is suggested the nature of the occupation is a factor which influences the extent of pesticide exposure. For this reason, the workers were put into 4 different job categories following the WHO standard protocol. The first are the “spray men”, workers who are directly involved in the preparation and application of pesticides. The mixers and loaders dilute and mix different pesticide formulations and make ready for application. The mode of application could be by aerial, ground or backpack means and “spray men” mostly spray pesticides by carrying manual or power operated machines in their shoulders. It is a common practice on all the farms for workers to alternate between pesticide mixing, loading and spraying activities so that all workers involved in such activities were considered as “spray men.” The other three occupational groups consisted of workers who were indirectly exposed. They include the pest assessors who inspect foliage residues and insect infestation before and after pesticide treatment; the tractor operators who drive tractors loaded with pesticide applying machines; and the spray machine mechanics who are involved in servicing and cleaning spray machines, tankers, hoses and other pesticide applying equipment. Pest control workers usually wear ordinary work clothes, consisting of long pants and long-sleeved cotton shirts. Gloved, masks or boots are not commonly worn during pest control activities. The remaining 161 subjects were unexposed subjects used as comparable controls. Most of them were industry workers with similar social and economic backgrounds. They had no known environmental or occupational exposure to OP and carbamate pesticides.

2. Blood sample collection

Five ml of blood sample was collected from each pest control worker from 2 September to 11 December, 1994, but no specific time was fixed for collecting blood samples from the control subjects. A questionnaire was administered to obtain information on selective OP poisoning symptoms, occupational histories such as job categories, the length of employment in pest control activities and variables that would alter baseline PChE levels. The questionnaire was prepared based on established procedures. Because ChE-inhibiting pesticides produce characteristic biologic effects, a fairly

<table>
<thead>
<tr>
<th>Pesticide trade name</th>
<th>Insecticide formulation</th>
<th>WHO toxicity category</th>
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<tbody>
<tr>
<td>Actelic</td>
<td>Actelic 50% EC</td>
<td>Moderately hazardous</td>
</tr>
<tr>
<td>Profenfor</td>
<td>Curacuron 250 EC/ULV</td>
<td>Moderately hazardous</td>
</tr>
<tr>
<td>Dimecron</td>
<td>Phosphanidon 250 ULV</td>
<td>Extremely hazardous</td>
</tr>
<tr>
<td>Dursban</td>
<td>Chloropyrifos 25 &amp; 48ULV</td>
<td>Moderately hazardous</td>
</tr>
<tr>
<td>Ultracide</td>
<td>Methidathion 40% EC</td>
<td>Highly hazardous</td>
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</tbody>
</table>

comprehensive range of symptoms was included in the questionnaire. The symptom dysgeusia was included to assess the extent of recall bias.

3. Biochemical tests

Cholinesterase determinations: PChE and AChE activities were determined based on the Michel\(^9\) electrometric method which depends upon the measurement of the acid produced by the action of ChE on acetylcholine (ACh). The acid produced was measured in terms of the change in pH produced by enzymatic activity in a standard buffer solution over a definite period of time. The same method was employed for PChE and AChE assay except for differences in the pH and concentration of the substrate and buffer solutions used.

After properly cleaning the skin, blood samples were drawn from veins in the inner forearms with a 5 ml venoject tube containing 7.2 mg of EDTA. Then plasma and red blood cells were separated by centrifugation at 2,000 rpm for 15 min.

In AChE determination, erythrocytes were subsequently washed with 0.9% NaCl solution. The volume of the cells was recorded, and the saline supernatant was removed to the point where the remaining volume of the cells and saline was twice the volume of the cells alone. After mixing, 0.4 ml of the cell suspension was hemolyzed in 9.6 ml of 0.01 saponin solution. One ml of the hemolyzed cell solution (representing 0.02 ml of the cells) was added to 1 ml of buffer solution containing 0.02M sodium barbital; 0.004M KH\(_2\)PO\(_4\); 0.6M KC\(_1\); pH is 8.1 at 25°C. This mixture was placed in a water bath at 25°C. After 10 min of equilibration, the initial pH (pH 1) was measured with a digital pH meter reading to the nearest 0.01. After adding 0.2 ml of 0.11M acetylcholine chloride solution, the reaction was allowed to proceed for 90 min and the final pH of the solution (pH 2) was determined. Finally, AChE activity was calculated and the necessary corrections made following the formula given by Michel\(^9\) and the results were expressed as \(\Delta \text{pH} / \text{ml/hr}\).

Similarly, PChE activity was assayed after diluting the plasma with water so that each ml of the solution contained 0.02 ml plasma. PChE activity was determined by mixing 1 ml of diluted plasma with 1 ml of the buffer solution (0.006M sodium barbital; 0.001M KH\(_2\)PO\(_4\) and 0.3 NaCl, pH 8.0 at 25°C and 0.2 ml substrate solution (0.1655M ACh chloride). Subsequent determination was carried out with the same reaction time and the activity calculated in the manner described for AChE. For each sample, PChE and AChE activities were determined twice and the average values were considered.

Liver function tests: Plasma AP and GPT values were estimated with commercially available diagnostic kits. In both cases, the kits contain ready made buffer solutions and substrates in the form of tablets. For AP assay, the test kit number 6391 (Bayer Diagnostics Company, Germany) was used according to the SCE\(^10\) recommendations. The method involves the continuous measurement of paranitrophenol at 405 nm formed from the hydrolysis of p-nitrophenylphosphate in a diethanolamine-magnesium chloride buffer solution. Similarly, test kit number 1442520 (Boehringer, Germany) was used for plasma GPT assay according to the IFCC recommendations\(^1\). In this method, the rate of NADH oxidation to NAD with prompt reduction of pyruvate was used to indirectly estimate the transaminase activity of GPT.

Statistical methods: All data were edited, coded and fed into an IBM-PC computer with a stored dBase IV program. The analysis was performed with the SPSS statistical package. In this study, \(P<0.05\), \(P<0.01\) and \(P<0.001\) were used as critical levels for statistical significance.

Results

The mean age of the exposed workers and controls was 32.8 (range 18–55) and 33.4 (range 17–51) respectively. Both population groups have similar age distributions. Personal details showed that smoking, chewing tobacco and alcohol consumption were common habits. Ninety-five (22.1%) of the exposed workers and 32 (19.9%) of the controls were smokers. Alcohol consumption was higher in the exposed group (23.9%) than in the controls (8.7%). Neither the exposed group nor the controls reported any history of hepatic disease or anaemia. Their history of medication showed that none of the study subjects had taken anti-cholinesterase drugs.

Intra-individual duplicate PChE and AChE values were within 1.8% and 2.3% in all cases, respectively. The coefficient of inter-individual variation among the controls was 22.4% for PChE and 29.3% for AChE activities. It is evident (Table 2) that the mean PChE and AChE activities in the exposed workers were significantly lower than in the controls (\(P<0.001\)). In addition, 55 (12.5%) and 49 (11.4%) of the exposed workers had values below 50% of the mean PChE and AChE activities of the controls, respectively. Furthermore, 58 (13.5%) and 47 (10.9%) of the exposed workers had PChE and AChE activities below the lower limits of the controls, respectively. On the other hand, no significant difference between the exposed
workers and the controls in AP and GPT values was noted (Table 2). Moreover, the mean AP and GPT values in the exposed workers and the control subjects were found to be within the recommended normal limits for these methods (50-250 U/l for AP, up to 29 U/l for GPT).

Table 2. The PChE & AChE activities and AP & GPT values for subjects, with corresponding ranges in parentheses (values are the mean ± SD)

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Control subjects (n = 161)</th>
<th>Exposed subjects (n = 410)</th>
</tr>
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<tbody>
<tr>
<td>PChE†</td>
<td>1.049 ± 0.235</td>
<td>0.949 ± 0.278*</td>
</tr>
<tr>
<td></td>
<td>(0.535 - 1.428)</td>
<td>(0.228 - 1.388)</td>
</tr>
<tr>
<td>AChE†</td>
<td>0.767 ± 0.231</td>
<td>0.639 ± 0.278*</td>
</tr>
<tr>
<td></td>
<td>(0.390 - 1.492)</td>
<td>(0.106 - 1.435)</td>
</tr>
<tr>
<td>AP†</td>
<td>77.59 ± 42.99</td>
<td>76.15 ± 29.93**</td>
</tr>
<tr>
<td></td>
<td>(33.90 - 390.90)</td>
<td>(32.90 - 176.20)</td>
</tr>
<tr>
<td>GPT†</td>
<td>14.24 ± 9.45</td>
<td>14.79 ± 10.67**</td>
</tr>
<tr>
<td></td>
<td>(5.00 - 90.00)</td>
<td>(5.00 - 110.00)</td>
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</tbody>
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Significance: *P<0.001, **Not significant. †Units= AChE: Δ pH/ml erythrocyte/hr, PChE: Δ pH/ml plasma/hr, AP: U/l, GPT: U/l.

Table 3. Cholinesterase activities of the exposed subjects by different job categories (values are the mean ± SD)

<table>
<thead>
<tr>
<th>Job categories</th>
<th>Number of workers (%)</th>
<th>PChE*</th>
<th>AChE*</th>
</tr>
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<tbody>
<tr>
<td>&quot;Spraymen&quot;</td>
<td>199 (46.3)</td>
<td>0.919 ± 0.297</td>
<td>0.548 ± 0.119</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.238 - 1.365)</td>
<td>(0.106 - 1.435)</td>
</tr>
<tr>
<td>Assessors</td>
<td>122 (28.4)</td>
<td>1.014 ± 0.256</td>
<td>0.720 ± 0.296</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.293 - 1.388)</td>
<td>(0.151 - 1.342)</td>
</tr>
<tr>
<td>Tractor operators</td>
<td>73 (16.9)</td>
<td>0.903 ± 0.267</td>
<td>0.712 ± 0.378</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.313 - 1.307)</td>
<td>(0.181 - 1.380)</td>
</tr>
<tr>
<td>Spray machine mechanics</td>
<td>36 (8.4)</td>
<td>0.992 ± 0.221</td>
<td>0.727 ± 0.282</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.320 - 1.315)</td>
<td>(0.186 - 1.292)</td>
</tr>
</tbody>
</table>

Significance: PChE "Spraymen" & Tractor operators vs. Assessors = p < 0.01. AChE "Spraymen" vs. Assessors, Tractor operators & Spray machine mechanics = p < 0.001.

The OP toxicity symptoms reported by the exposed workers are indicated in Table 5. Although their distribution is different, almost all the symptoms were reported by the exposed workers. The symptom dysgeusia, which was included as a control was not reported at all. This result suggests the absence of recall bias among the pest control workers. Most of the symptoms reported were transient in nature and workers were relieved from such complaints whenever they had a break from continuous pesticide exposure. The main symptoms by order of importance were; headache, weakness, sweating, wheezing and blurred vision. Evidence of excessive cholinergic activity such as myosis, muscle weakness and tremor were also reported.
The association of these symptoms and ChE activity depression were assessed by comparing the mean AChE values for the exposed workers who reported these symptoms with those for workers who were not exposed (Table 5). From this table, a definite relationship can be observed between the reported symptoms and AChE activities. Although the differences are not statistically significant, the mean AChE activities for workers with the various reported symptoms were lower than for those without these symptoms. But of all the complaints of possible ChE-related symptoms, a significantly lower AChE activity was observed only in those who reported sweating as compared to those who did not (P < 0.05).

Discussion

The molecular basis of the action of OPs on neural influx transmission is related to the inhibition of AChE within the central and peripheral nervous system, promoting the accumulation of ACh at the nerve synapse. Monitoring the effects of groups of compounds with specific methods well established for OPs is independent of their chemical structures, so that depression of plasma and/or red blood cell ChE activity is the most satisfactory and generally available evidence of excessive absorption of OP pesticides. Although determination of PChE is used because of its simplicity, both AChE and PChE are preferable, because OPs preferentially inhibit either of the two enzymes. In the
The mean PChE and AChE values observed in the control group were more or less comparable with those reported by Abiloa et al. and Brown et al. The levels of PChE were higher than the levels of AChE both in the OP exposed workers and controls. Such observations are not typical of this study and similar results were noted by Rider et al. and Brown et al. There is no correlation between AChE and PChE values in the absence of ChE inhibition and PChE is highly influenced by several factors among which sex, age, race and seasonal variation are some of the most important ones. The high level of PChE both in the control and OP exposed workers may be due to the fact that all the study subjects were males. Because serum ChE activity is significantly higher in males than in females. Season as a factor may also be a cause of the observed difference, because human PChE is also thought to be influenced by seasonal variation, with the levels being higher in summer than in winter. Moreover, PChE is also influenced by race or ethnic variation, although it is not known whether this is the result of genetic or nutritional factors. The intra-individual differences observed between duplicate ChE readings were lower than the values reported by Quinones et al. Findings in the present study therefore showed a better match between the duplicate readings. The coefficient of inter-individual PChE variation observed among the control subjects was lower than the variations observed for AChE activities. This is against to the opinion that in normal persons the PChE is more variable than AChE and is less an indicator of exposure to ChE-inhibiting pesticides. Blood ChE activity varies both at individual and group levels. The common existence of wide inter-individual variation in both PChE and AChE activities among normal subjects has also been described by Coye et al. and Misra et al.

The significant differences observed between the exposed workers and the controls in both PChE and AChE activities (Table 2) suggest that the pest control workers are experiencing depressed ChE activities as a result of occupational exposure to the OP insecticide formulations used in the state farms. In agreement with this, the work of Ciesielski et al., Quinones et al., Misra et al., Gupta et al. demonstrated similar results. Unlike these findings, in other studies no significant changes in PChE or AChE activities among OP exposed farm workers have been observed. A slight increase in ChE levels after OP exposure have also been reported. Variations in ChE activities following exposure to OPs is suggested to be due to the differences in the extent of exposure to various OPs and in the method of analysis of blood samples. The wide range of ChE activity levels in normal subjects may also account for such differences. For such reasons, the importance of obtaining pre-exposure baseline measurements for the surveillance of potentially exposed workers is strongly recommended.

The absence of significant differences between the exposed workers and controls in plasma AP and GPT levels (Table 2) suggests that there was no marked impairment of liver function at this level of exposure. This result agrees with the findings of Misra et al. and Gupta et al. In contrast to this, significantly higher AP and GPT levels in pesticide exposed subjects compared to the control groups were also reported, but the subjects of most of these studies were exposed to a number of pesticides besides OPs.

Significant reductions in PChE and AChE activities among the “spray men” suggest that these workers had greater OP exposure than the workers in the other occupational groups. That the “spray men” are the highest risk group may be due to the fact that they have frequent contact with the most concentrated pesticide formulations during mixing, loading, flagging and application. Similarly, Spigel et al. observed a significant degree of exposure in applicators and warehouse handlers of pesticides compared to farmers in others job categories. A higher level of OP pesticide metabolites in urine was also noted in mixers and applicators than in mechanics and other field workers. Tractor operators were the second most affected group as shown by a significantly lower PChE activity than in pest assessors (Table 3). From field studies, it was observed that tractor operators mainly operate tractors loaded with pesticide applicating machines and always stay near the spray area to drive tractors from treated to untreated rows. They also sometimes help “spray men” in carrying the hose and operating the spray gun, so that tractor operators may be exposed to about the same dose of pesticide as the “spray men” are.

The length of employment as a pest control worker was found to affect ChE activity. This was demonstrated by relatively lower AChE but not PChE activity in workers exposed for 5 years or less than in those with longer years of exposure (Table 4). This result was in contrast to the idea that workers exposed to ChE inhibitors over several years may demonstrate long-term ChE activity.
depression23). But, in close agreement with this study, Aguilar et al.24 observed significant PChE and AChE activity depression in agricultural workers in Bolivia who had been using pesticides for 1 to 5 years compared to those with longer years of exposure. It can therefore be suggested that exposed workers most affected are those who have been exposed to pesticides for less than 5 years, who have little experience in pesticide handling. It is also suggested that excessive pesticide absorption occurs more likely in new employees than in those who through experience have come to minimize their exposure3.

The occurrence of toxicity symptoms in OP exposed populations have been reported by many investigators. For instance, earlier studies by Ciesielski et al.23, Quinones et al.15, Aguillar et al.2, and Wu et al.25 demonstrated the occurrence of one or more of these symptoms, with weakness and headache being reported more frequently. On the other hand, Jeyaratnam et al.26 and Sansur et al.27 noted no symptom or clinical manifestation of poisoning in OP exposed farm workers.

Although symptoms other than sweating were more frequently reported by exposed workers, others had no association with a significant reduction in AChE activities (Table 5). Therefore, in the present study, only this symptom may be substantiated in terms of reduced AChE activity. Erythrocyte ChE has been reported to closely parallel the level of ChE in the central and peripheral parasympathetic nervous system and is considered to be the best indicator of ChE activity in the nervous system tissue8. Thus inhibition of erythrocyte ChE activity can be correlated with the effects on the nervous system28. Several studies have associated ChE activities with signs and symptoms which are commonly known to appear due to OP intoxications. The frequent occurrences of poisoning symptoms accompanied by a significant depression in ChE activities have been shown by Amr et al.22 and Loosli29. In contrast, the work of other investigators15, 17, 19, 24 showed no significant relationship between the reported symptoms and depressed whole blood, plasma or erythrocyte ChE activities. Still others2, 25 noted only minimal association between ChE activities reduction and the reported morbidity. The absence of a strong association between the reported symptoms and ChE activity reduction may occur for several reasons. The appearance of symptoms depends more on the rate of fall in ChE activity than the absolute level of ChE reached3. Signs and symptoms of OP poisoning occur when more than 50% of PChE and AChE activity is inhibited. In addition, the wide fluctuation in PChE and AChE levels in normal persons may be the cause of these differences3, 15, 26. Although no evidence was found in the present study, under-reporting and/or over-reporting of symptoms due to workers recall bias are also suggested to be the causes of discrepancies between the prevalence of cholinergic symptoms and the degree of ChE activity reduction expected.

Conclusion

Pest control workers are generally at risk of OP pesticide related problems as shown by significant depression in both plasma and erythrocyte ChE activities. The health risks are more apparent in "spray men" and in those who may have little experience in the safe handling of pesticides. The symptoms which are frequently reported by farm workers are also important and should not be neglected by physicians, although their association with significant AChE depression has not been observed in the present study.

Pre-employment medical examinations including ChE determinations are important to rule out problems arising from the naturally wide ChE activity variations among non-exposed individuals. These baseline ChE activity values could therefore be used as references for follow up of the ChE status of farm workers after OP pesticide exposure. Adequate periodic medical examinations, focusing on the most high risk groups such as the "spray men" also help in the early detection of health impairment in exposed workers. Promoting workers' awareness of the hazards from the use of pesticides through pre-placement orientation of newly employed workers in particular and thorough on-job training of pest control workers in general should be emphasized.

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References