Erythrocyte cholinesterase activity levels in desert farm workers

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In this study we have examined 532 migrant farm workers engaged mainly in the cultivation of vegetable crops, in both greenhouses and open farms, and an equal number of controls. Erythrocyte acetylcholinesterase activity (AChE) was measured to determine the degree of toxicity due to exposure to organophosphate and carbamate pesticides in the farm workers employed either as foremen (41.5%) or farmers (58.5%). The mean ages of the farm workers and controls were 35.2 ± 7.4 (mean ± SD) years and 34.6 ± 7.1 years. AChE activity of the farm workers and controls was 3.89 ± 0.64 IU/ml (mean ± SD) and 4.15 ± 0.29 IU/ml. The haemoglobin adjusted erythrocyte cholinesterase activity (HACHe) was 29.96 ± 4.14(mean ± SD) for farm workers and 32.10 ± 2.26 for controls. AChE activity was very highly significantly lower for the foremen (3.76 ± 0.69) compared to farmers (3.98 ± 0.59) (Student's t-test = 4.13, p = 0.0001). HACHe was also very highly significantly lower for foremen (29.24 ± 4.37) compared to farmers (30.46 ± 3.88) (Student's t-test = 3.64, p = 0.0001). The poorly controlled use of pesticides in the farms appeared to have caused sub-clinical intoxication in the farm workers and indicated the need for training and implementation of hygiene practices.

Key words: Agricultural workers; carbamates; erythrocyte acetylcholinesterase; farm workers; greenhouses; haemoglobin, haemoglobin adjusted erythrocyte cholinesterase, open farms, organophosphates; pesticides

INTRODUCTION

Occupational exposure to pesticides has been shown to be significant in at least two groups of workers: farm workers and workers at pesticide production factories. The main route of exposure in unprotected farm workers has been by inhalation, transdermally and by ingestion.1-4 Morbidity patterns in farm workers have been identified as headache, weakness, abdominal pain, ataxia and anorexia.5-6 Organophosphates and carbamates inhibit acetylcholinesterase which causes accumulation of acetylcholine at nerve endings resulting in a cholinergic or hypersecretory syndrome.7 Organophosphate pesticides produce irreversible inhibition because of phosphorylation of the enzyme, and it may take 4-6 months for acetylcholinesterase to return to normal levels. Carbamates cause reversible inhibition by physically blocking the active receptor sites but this effect may be rectified in 48-72 hours.6,7 Farm workers have been identified as a high risk group for occupational poisoning. Wasseling et al.8 reported that cholinesterase inhibitors caused 71% of the reported occupational accidents, 63% of hospitalizations and 36% of deaths in Costa Rica. Coye et al.9 identified mixers, loaders and application operatives working in farming and public health areas as high risk groups. Exposure to pesticides poses a significant risk in rural farming populations of developing countries in particular, because the users cannot understand pesticide packing labels and colour codes, are not persuaded to follow any instructions and are not trained in farm hygiene or in using protective equipment.1,2 In developing countries like the UAE, these workers are mostly expatriates from third world countries and tend to be socio-economically disadvantaged.
Beliefs that pesticides are toxic and can cause health hazards are not very common among these farm workers because of their insufficient training. Those who would use protective equipment if provided are reluctant to demand such protection or even seek medical treatment because of the fear of losing their jobs and being deported to their home country.

There have been few research studies of farm workers occupationally exposed to pesticides, and hence data on acute and chronic health effects related to their toxic exposures are lacking. Increased efforts are needed to study chronic exposure effects, particularly neurobehavioural effects, long term neurological dysfunction and other symptoms and signs compatible with cholinesterase inhibition. A dose response relationship between exposure and cholinesterase depression has been reported by Rama and Jagat and Yamanaka et al. Acetylcholinesterase activity (AChE) in the red blood cells and butyrylcholinesterase activity in plasma have been used to monitor the extent of organophosphate and carbamate exposure. However, Coye et al. suggested inhibition of AChE as the better indicator of biological effects. The use of AChE as a useful biomarker for chronic pesticide toxicity has been reported by Walker, Meuling et al. and McConnell and Magnotti. London et al. reported excellent sensitivity and specificity for AChE using a spectrophotometric field kit.

Farming is a new industry in the UAE and is rapidly growing, with the farm employees being migrant workers, most of whom have low educational attainments and possess little training in the use of pesticides or other farm practices. The study described in this paper was undertaken in an effort to determine the pattern of AChE and HAcHE levels from low dose, chronic exposure in the farm workers. The determination of pre-exposure enzyme levels was not feasible because crops are grown both in summer and winter seasons. Therefore, we compared the pattern of the post-exposure enzyme activity in two populations exposed to pesticides at different levels and also in controls who are supposed to have had no occupational exposure. We have used the acetylcholinesterase enzyme activity in the control population as the reference activity and have attempted to determine the level of occupational exposure by using AChE and HAcHE as biological markers.

**MATERIALS AND METHODS**

Multi-stage sampling technique was used to sample five of the twenty-five sampling areas, and in each of these areas one-fifth of the farms were included in the study. The control population was selected from the expatriate workers attending the labour clinic routinely to certify their good health so that they could renew their work permit and who were not occupationally exposed to pesticides, i.e. had never worked in farms or pesticide related industries. The exposed (farmers and foremen) and unexposed (controls) populations were matched for nationality, age and socio-economic status (salary and living conditions). The response rate of the study population was 100%.

The study population consisted of 221 farms comprising both open farms (84.1%) and greenhouses (15.9%), and the sampled employees included 221 (41.5%) foremen and 311 (58.5%) farmers and these two categories formed subgroups by probable degree of exposure. The foremen conducted day-to-day management of the farm and delegated duties to the farmers; they were also responsible for stocking the pesticides, preparing the diluted mixtures for spraying and spraying the pesticides. The farmers, on the other hand, undertook labouring jobs which included tilling the soil, harvesting the produce, giving occasional help to the foremen in spraying the pesticides and cleaning and putting aside the implements used in spraying; the involvement of the farmer in handling pesticides depended on the foremen and these duties were allocated only occasionally.

A specifically designed questionnaire was applied to those farm workers who agreed to participate in the study. A 10ml capillary blood sample was then collected from these workers to estimate AChE, HAcHE and haemoglobin levels. A Testmate Cholinesterase kit (EQM Research, Cincinnati, OH, USA) was used to analyze the capillary blood for haemoglobin and enzyme activity level. Blood pressure was measured using a close fitting cuff and aneroid sphygmomanometer. Body Mass Index (BMI) was calculated using the formula BMI = wt/h², where weight is measured in kilograms and height in meters.

The data were analyzed using Statistical Package for Social Sciences (SPSS) for Windows version 6.1. Analysis of data included computations of statistical parameters and comparisons of the sample means using the Student's t-test. Inter-group differences were measured using Analysis of Variance (ANOVA), and linear regression was used to find the association of AChE and HAcHE independently with period of exposure (length of service in Al-Ain), type of job (categorized as foreman, farmer or control) and area of work (categorized into different farming areas), type of farm (categorized into greenhouse or openfarm), nationality (Bangladeshi, Egyptians, Indians, Pakistanis and Others), systolic and diastolic blood pressures and BMI.

**RESULTS**

The population of 532 farm workers and 532 controls comprised Bangladeshis (38.1%), Egyptians (27.8%), Indians (12.9%), Pakistanis (16.5%) and Others (3.8%) (Iranis, Syrians, Palestinians, Sudanese and Sri Lankans). The mean ages of the farm workers and controls were 35.2 ± 7.4 and 34.6 ± 7.1 years respectively. The mean systolic blood pressure (mm Hg) of
the farm workers and controls were 117.5 ± 8.2 and 117.3 ± 13.49 and the mean diastolic blood pressures were 78.1 ± 8.2 and 78.9 ± 11.3 respectively. The mean haemoglobin levels (g/dl) for the exposed and unexposed were 12.86 ± 1.47 and 12.93 ± 0.83, the mean AChE (Ul/ml) were 3.89 ± 0.64 and 4.15 ± 0.29 and HACHe (Ul/g haemo.) were 29.96 ± 4.14 and 32.10 ± 2.26.

Many workers had been previously employed as farm workers such that the total period of work in their home country and in the UAE as farm workers was 5.33 ± 2.26 years for foremen and 3.12 ± 2.6 years for farmers and 4.12 ± 4.7 years for controls, while in the UAE alone their mean periods of work were 3.7 ± 3.7 years and 1.9 ± 1.6 years for foremen and farmers respectively. Table 1 shows age, years of exposure and enzyme activity levels for foremen, farmers and controls. AChE, haemoglobin and HAChE levels in foremen (i.e. the most exposed group) were statistically significantly lower than in the controls.

Demographic characteristics and enzyme differences between the farm workers working in greenhouses and open farms did not show any statistical significance. Internationality differences for AChE activity, haemoglobin and HACHe and years of exposure are shown in Table 2. The differences for AChE and HACHe were statistically significantly different for foremen, farmers and controls among the five nationality groups. Indian foremen, farmers and controls showed least enzyme depletion while Egyptian foremen and farmers showed the highest.

The farms in each area showed considerable homogeneity in terms of crops, type of soil, type of pests, type of pesticides and frequency of pesticide usage, but wide inter-area differences were noted. The types of pesticides used also varied from area to area, within the same generic groups of organophosphates, carbamates, pyrethroids and organochlorines. The frequency of pesticide usage and the dilution ratios also differed in different areas depending on the prevalence of the pest and the type of crop currently under

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**Table 1. Age, years of service and enzyme pattern among farm workers and controls**

<table>
<thead>
<tr>
<th></th>
<th>Foremen Mean</th>
<th>Farmers Mean</th>
<th>Controls Mean</th>
<th>95% CI for diff</th>
<th>95% CI for diff</th>
<th>95% CI for diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>40.53</td>
<td>31.37</td>
<td>34.58</td>
<td>10.08–8.24</td>
<td>5.19–6.71</td>
<td>−4.22–−2.21</td>
</tr>
<tr>
<td>Years of service (yrs)</td>
<td>5.33</td>
<td>3.12</td>
<td>4.12</td>
<td>−2.65–−1.77</td>
<td>0.69–1.72</td>
<td>−1.50–−0.52</td>
</tr>
<tr>
<td>AChE (Ul/ml)</td>
<td>3.76</td>
<td>3.99</td>
<td>4.15</td>
<td>0.12, 0.34*</td>
<td>−0.49–−0.28*</td>
<td>−0.23–0.09*</td>
</tr>
<tr>
<td>Haemoglobin (g/dl)</td>
<td>12.78</td>
<td>13.08</td>
<td>12.93</td>
<td>0.04, 0.57*</td>
<td>−0.39, 0.09*</td>
<td>−0.00, 0.30*</td>
</tr>
<tr>
<td>HACHe (Ul/g)</td>
<td>29.19</td>
<td>30.51</td>
<td>32.10</td>
<td>0.59, 2.0*</td>
<td>−3.52–−2.29*</td>
<td>−2.07,−1.12*</td>
</tr>
</tbody>
</table>

* p-value < 0.0001

**Table 2. Enzyme characteristics and years of service for foremen, farmers and controls according to their nationalities**

<table>
<thead>
<tr>
<th></th>
<th>AChE* (Ul/ml) Mean (Median) 95% CI</th>
<th>Haemoglobin (g/dl) Mean (Median) 95% CI</th>
<th>HACHe* (Ul/g) Mean (Median) 95% CI</th>
<th>Exposure (yrs) Mean (Median) 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bangladesh</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Foremen (n=54)</td>
<td>3.99 (3.94) 3.84–4.14</td>
<td>12.72 (12.8) 12.42–13.02</td>
<td>31.20 (31.4) 30.29–32.11</td>
<td>4.93 (5.0) 4.40–5.45</td>
</tr>
<tr>
<td>Farmers (n=153)</td>
<td>4.06 (4.04) 3.96–4.16</td>
<td>12.98 (13.0) 12.78–13.19</td>
<td>31.19 (31.4) 30.53–31.85</td>
<td>2.83 (2.0) 2.44–3.21</td>
</tr>
<tr>
<td>Controls (n=207)</td>
<td>4.16 (4.20) 4.11–4.20</td>
<td>12.88 (12.9) 12.75–13.01</td>
<td>32.20 (32.5) 31.83–32.58</td>
<td>2.85 (2.0) 2.16–3.54</td>
</tr>
<tr>
<td>Egyptians</td>
<td></td>
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<tr>
<td>Foremen (n=109)</td>
<td>3.69 (3.64) 3.56–3.83</td>
<td>12.97 (13.0) 12.58–13.35</td>
<td>28.03 (28.3) 27.13–28.93</td>
<td>5.39 (5.0) 4.86–5.91</td>
</tr>
<tr>
<td>Farmers (n=39)</td>
<td>3.79 (3.83) 3.62–3.95</td>
<td>13.20 (13.0) 12.80–13.60</td>
<td>28.99 (28.7) 27.92–30.06</td>
<td>4.77 (4.0) 3.68–5.86</td>
</tr>
<tr>
<td>Controls (n=148)</td>
<td>4.33 (4.30) 4.25–4.40</td>
<td>13.10 (13.0) 12.75–13.44</td>
<td>33.20 (32.7) 32.67–33.73</td>
<td>0.87 (0.0) 0.03–0.17</td>
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<tr>
<td>Indians</td>
<td></td>
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</tr>
<tr>
<td>Foremen (n=12)</td>
<td>4.03 (3.73) 3.58–4.48</td>
<td>13.09 (13.0) 12.17–14.01</td>
<td>30.95 (30.6) 29.21–32.69</td>
<td>4.92 (5.0) 3.32–6.51</td>
</tr>
<tr>
<td>Farmers (n=57)</td>
<td>4.09 (4.01) 3.96–4.23</td>
<td>13.59 (13.7) 13.29–13.90</td>
<td>30.17 (29.7) 29.37–30.97</td>
<td>2.49 (2.0) 2.00–2.98</td>
</tr>
<tr>
<td>Controls (n=69)</td>
<td>4.15 (4.18) 4.11–4.19</td>
<td>13.01 (12.9) 12.88–13.14</td>
<td>31.99 (31.9) 31.66–32.32</td>
<td>5.23 (4.0) 4.59–5.86</td>
</tr>
<tr>
<td>Pakistanis</td>
<td></td>
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<tr>
<td>Foremen (n=39)</td>
<td>3.55 (3.75) 3.32–3.77</td>
<td>12.23 (12.5) 11.66–12.80</td>
<td>29.13 (29.5) 27.80–30.47</td>
<td>5.59 (5.0) 4.99–6.19</td>
</tr>
<tr>
<td>Farmers (n=49)</td>
<td>3.80 (3.65) 3.63–3.97</td>
<td>12.88 (12.8) 12.60–13.16</td>
<td>29.57 (29.2) 28.49–30.64</td>
<td>3.78 (3.0) 2.92–4.63</td>
</tr>
<tr>
<td>Controls (n=86)</td>
<td>4.12 (4.08) 4.07–4.17</td>
<td>12.87 (12.8) 12.76–12.98</td>
<td>31.95 (31.9) 31.59–32.30</td>
<td>4.32 (3.0) 3.52–5.11</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmers (n=13)</td>
<td>3.91 (3.79) 3.62–4.21</td>
<td>12.47 (12.2) 11.74–13.20</td>
<td>32.01 (34.4) 29.28–34.74</td>
<td>1.77 (2.0) 1.21–2.33</td>
</tr>
<tr>
<td>Controls (n=20)</td>
<td>4.02 (3.95) 3.89–4.16</td>
<td>12.48 (12.6) 11.99–12.97</td>
<td>32.40 (31.9) 31.17–33.62</td>
<td>3.18 (0.0) 0.48–6.85</td>
</tr>
</tbody>
</table>

* p < 0.01; ** p < 0.001
cultivation. The type of crops varied from area to area except for one or two common crops. Multiple regression analysis, with AChE as the dependent variable and covariates significantly associated with pesticide exposure as independent variables, showed that farming area (pesticides usage), type of job (intensity of exposure), nationality, period of exposure and type of farm were very significant predictors of AChE activity depletion (Table 3). Age was a nonsignificant contributor to the multiple regression model. When a similar model was computed for HAcHE, with HAcHE as dependent variable and all the covariates of AChE as independent variables, pesticides usage and intensity of exposure were very highly significant predictors, while period of exposure was a nonsignificant \( p = 0.06 \) contributor to the model.

DISCUSSION

Farm workers described in this study lived on the farm and their diet consisted almost entirely of the produce of the farm. Most farms did not have a separate or isolated room to store fertilizers, pesticides and farm equipment and so these were stored either in a room adjacent to the living quarters or, in some instances, in the living area itself. Most of the farm workers kept a set of clothes to use on the farm during work while others used their casual clothes and even went to sleep wearing these clothes. The majority of the workers did not use any protective equipment, not even shoes, while some used gloves during harvesting. As has been found in other studies\(^\text{3}\) the farm workers did not complain of the lack of protective equipment because they were unaware of the dangers of the different types of exposure to pesticides. The farm workers had not been trained in constituting pesticides nor in spraying, and their minimal educational achievements prevented them from being able to read instructions or decipher any colour codes on the pesticide packages. As with other studies\(^\text{3}\) there was no belief among these workers that pesticides are toxic and cause health problems; hence these workers did not take any precautions while handling pesticides, thereby maximizing their exposure. The controls were exposed to pesticides only in the form of food residues and, less probably, as drift from pesticide spray if this occurred in the area in which they resided. The significant differences among farm workers and controls reflected the differences in exposure to pesticides, all else being similar.

AChE and HAChE levels were significantly lower in farm workers compared to the control population than in nonfarm workers; a dose response relationship was evident in the lowest values being found among those involved most frequently in applying pesticides, i.e. foremen. Similar findings have been previously reported\(^\text{18,23}\) with foremen who handled cholinesterase inhibiting pesticides for a longer period and more intensely than farmers and who experienced greater reductions in AChE activity. These findings are also supported by the work of Lopez-Carillo and Lopez-Cervantes,\(^\text{24}\) Coye \textit{et al.},\(^\text{25}\) McConnell \textit{et al.}\(^\text{4}\) and Faustini \textit{et al.}\(^\text{25}\) The depletion of AChE reported in the UAE workers occupationally exposed to cholinesterase inhibiting pesticides correlated well with the frequency of usage and length of use, thereby confirming similar findings elsewhere\(^\text{12,26,27}\) which have reported that the depletion of cholinesterase activity was proportional to the type of pesticides used and period of exposure. Brown \textit{et al.},\(^\text{28}\) Spiegl \textit{et al.}\(^\text{26}\) and Lopez-Carillo and Lopez-Cervantes\(^\text{24}\) reported that fluctuations in cholinesterase depletion corresponded with changes in the use of organophosphate pesticides during the farming season.

In summary, the findings reported in this paper have confirmed that the use of cholinesterase inhibiting pesticides on the farms caused the depletion of AChE and HAChE in the farm workers. The depletions in AChE and HAChE levels were strongly influenced by the area of work. The type of pesticides used in different areas varied, as did also the amount used and the frequency of use; this pattern of pesticide usage was reflected by significant differences in AChE and HAChE patterns between the workers in different areas. Farm workers in farming area Hayer showed the highest depletions while Slimate the lowest, which

\begin{table}
\centering
\caption{Significance of independent variables in multiple regression analysis}
\begin{tabular}{|l|l|l|l|l|l|}
\hline
Dependent variable & Independent variable & \( B \) & \( SE \ B \) & \text{Beta} & \( t \) & \( p \text{-value} \) \\
\hline
AChE & Pesticide usage & 0.100 & 0.0133 & 0.303 & 7.57 & 0.0001 \\
 & Intensity of exposure & 0.066 & 0.0325 & 0.095 & 2.05 & 0.05 \\
 & Nationality & 0.033 & 0.0147 & 0.071 & 2.25 & 0.05 \\
 & Period of exposure & 0.017 & 0.0068 & 0.091 & 2.59 & 0.01 \\
 & Haemoglobin & 0.212 & 0.0128 & 0.497 & 16.54 & 0.0001 \\
\hline
HAcHE & Pesticide usage & 0.949 & 0.1010 & 0.432 & 9.41 & 0.0001 \\
 & Intensity of exposure & 0.779 & 0.2470 & 0.169 & 3.16 & 0.001 \\
 & Period of exposure & 0.097 & 0.0516 & 0.075 & 1.87 & 0.06 \\
\hline
\end{tabular}
\end{table}

\( B \) = Slope of regression line
\( SE \ B \) = Standard error of \( B \)
\text{Beta} = Standardized regression coefficient
\( t \) = Student's \( t \)-test
\( p \) = Two tailed observed significance level
correlated well with the higher per dunnum (1,000 m$^2$) usage in Hayer than in Sleimat. The type of job also contributed significantly in depleting AChE and HAcH levels as evidenced by foremen showing lower values compared to farmers and farmers showing lower values compared to controls. Nationality and period of exposure significantly predicted AChE depletion and nonsignificantly HACHe. Covariates of both AChE and HACHe suggested that the persistent usage of pesticides without any protective equipment and also without implementation of farm hygiene might eventually affect the health of these farm workers. Our findings support similar reports by other investigators. It is clear that education and training of farm workers in handling pesticides in an appropriate way and use of protective equipment are needed to reduce their exposure and hence the long term risks to their health.

REFERENCES