Relationship Between Cholinesterase Inhibitor Insecticides In Human Blood And Insecticides Residues In Drinking Water In Mansoura City- Egypt
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Abstract
Pesticides are classified into four major groups; insecticides, herbicides, rodenticides and fungicides. Insecticides poisoning is one of the most common poisoning affecting our population. The study investigates the relationship between cholinesterase inhibitor insecticides in human blood and insecticides residues in drinking water in Mansoura city. Sixty patients suffering from insecticides poisoning admitted to Emergency Hospital Mansoura University- Egypt in March 2005, were included in this study. Blood samples were collected from these patients. The studied patients and their relatives were asked to obtain water samples of their drinking water from the localities where they live. Thirty drinking water samples were collected. Blood and water samples were extracted using Liquid-liquid extraction technique (LLE) and analyzed for pesticides using Gas Chromatography-Mass Selective Detector (GC-MSD). In addition, blood samples were analyzed for plasma pseudo-cholinesterase level (PChE) and red blood cells acetyl cholinesterase activity (AChE). The analysis of drinking water and blood samples revealed organophosphorous (Mevinphos) and organochlorine (Aldrin) insecticides. Moderate insecticides poisoning was more common among the studied groups (65.0%) compared to mild and severe poisoning. The peak incidence of poisoning was in the age group from 20-30 years (46.67%), followed by the age group from 10-20 years (43.33%). There was a female sex predominance among the poisoned patients (56.67%). There was a negative correlation between the severity of poisoning and each of PChE levels and AChE activity % being lowest (p<0.001) in severe poisoning compared to moderate and mild poisoning.

INTRODUCTION
Drinking water resources in Dakahlya governorate are either surface from Nile River and the related irrigation canals or subsurface resources raised from some boreholes drilled in the Quaternary aquifer at Mit-ghamr, Aga and Sinbillawin districts. Abd el-Daiem and Ramsussen (1) reported that at Mit-ghamr, the surface water level was almost +9 m where it was about +6 m for the surrounding groundwater level. Herein there is downward movement of the surface water to recharge the groundwater from the influent stream in this sector. On the contrary, at Mansoura, the surface water level in the Nile branch is +1.8 m, while it is about +4 m in the nearby water wells, where the river behaves as a drain.

It is recognized that the environmental degradation products of pesticides may be a problem in drinking water ( 21 , 4 ). Acute poisoning by pesticides is common; it usually occurs due to careless use, misuse or occupational exposure (18). According to EEC (10) and WHO (20), the presence of toxic pollutants in water especially insecticide chemicals can change the quality of surface water. In order to monitor the quality of surface water, samples must be analyzed periodically to assess the residues levels and distribution of chemical pollutants in the surface water.

Insecticides residues determination in water requires analysis at or below ppb level ( g/l) (17), so the investigation of water pollution with such low concentration requires a concentration step prior to the analysis. Concentration step is often necessary to raise the concentration of the insecticide chemicals (found in the water at trace levels) above the sensitivity levels of the detection of the analytical tools. Liquid-liquid extraction technique (LLE) has been considered as an effective method for concentration of trace organic pollutants including insecticides from water samples (21).

The aim of the present work is to investigate the relationship between cholinesterase inhibitor insecticides in human blood
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and insecticides residues in drinking water in Mansoura city.

SUBJECTS AND METHOD

Sixty patients were admitted to the Poison Unit at Emergency Hospital, Mansoura University-Egypt during March 2005. Patients were presented with signs and symptoms of cholinesterase inhibitor insecticides poisoning, with a variant degree of severity including sweating, diarrhoea, vomiting, excessive salivation, miosis, breathing problems , heart dysrhythmias and extreme anxiety. These patients were enrolled in our study. A history was taken from the patients through answering a questionnaire, which included; age, sex, route and mode of exposure to the insecticides.

Blood samples were collected from all patients (n=60). The studied patients and their relatives were asked to obtain water samples of their drinking water from the localities where they live. Thirty drinking water samples were collected. The blood and water samples were analyzed for determination and identification of pesticides by GC-MSD. In addition, the blood samples were investigated for the levels of pseudo-cholinesterase enzyme and acetyl cholinesterase activity by a colorimetric method.

Extraction of water and blood samples; (11):

- pH of each sample (10 ml) was adjusted to 7 by adding solid sodium bicarbonate.
- Each sample was extracted with 5 ml of methyl tertiary-butyl ether for 5 minutes using a rotary mixer.
- Samples were left to stand for 5 minutes, then the upper layers were taken and re-extracted with a second 5 ml portion of methyl tertiary-butyl ether.
- The two extracts of each sample were filtered through a Wattmann filter paper No 4 into a clean tube and the extract of all samples were evaporated to dryness under compressed air.

Residues of the evaporated extracts were reconstituted by 100 µl chloroform. 1 µl of each extracted sample was injected into GC-MSD (Hewlett Packard 6890 series) of ECD (Electron Captured Detector) under the following conditions, (14):

- Carrier gas (He)
- Capillary column; model No: HP19091Z-102, Hp-1 Methy Siloxane, length 25m, diameter 200 µm, film thickness 0.33µm.
- Flow rate 1.0 ml / min, velocity 39cm / sec, Mode: split less,
- Pressure 16.1 psi, Run Time 12.5 min
- Thermal Aux 2 (MSD; Mass Spectrum Detector), Temp 280 °C, Max.temp. 325 °C, Oven Temp 200 °C.

Determination of pseudo-cholinesterase enzyme levels and acetyl cholinesterase enzyme activity of blood samples:

Levels of pseudo-cholinesterase enzyme (PChE) and acetyl cholinesterase (AChE) activity were analyzed by spectrophotometer according to Ellman et al., (9) and Crane et al., (6), respectively.

STATISTICAL ANALYSIS

The statistical analysis of data was done by using Excel and SPSS programs statistical package for social science version 10. The description of the data was done in the form of mean (+/-) SD for quantitative data and frequency & proportion for qualitative data. The analysis of the data was done to test statistical significant difference between groups. For quantitative data, one way ANOVA test was used to compare more than 2 groups, and for qualitative data Chi-square test was used. (N.B:P is significant if < or = 0.05 at confidence interval 95%).

RESULTS

The analyzed drinking water and blood samples revealed the presence of organophosphorous (Mevinphos) and organochlorine (Aldrin) insecticides. The mass spectra of fragment ions are: 127, 192, 109 for Mevinphos (fig. 1) and 66, 263, 79, 91, 101 m/z for Aldrin (fig. 2).
Table (1) showed that the peak incidence of poisoning by cholinesterase inhibitor insecticides was in the age group from 20-30 years (46.67%) (suicidal mode of poisoning was common among this group particularly in females), followed by the age group from 10-20 years (43.33%).
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Figure 3
Table 1: Age-related incidence of acute cholinesterase inhibitor insecticide poisoning

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Poisoned patients (n)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-20</td>
<td>26</td>
<td>43.33</td>
</tr>
<tr>
<td>20+ -30</td>
<td>28</td>
<td>46.67</td>
</tr>
<tr>
<td>30+ - 40</td>
<td>3</td>
<td>5.0</td>
</tr>
<tr>
<td>40+ -50</td>
<td>3</td>
<td>5.0</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Figure 3 showed that there was a female sex predominance among the poisoned patients (56.6%).

Figure 4
Figure 3: Acute cholinesterase inhibitor insecticides poisoning as regard the sex predominance

Table (2) showed that the moderate poisoning of cholinesterase inhibitor insecticides (according to cholinesterase enzyme levels) was more common among the studied group (65.0%) compared to mild and severe poisoning.

Table 2: Patients (n=60) of acute cholinesterase inhibitor poisoning as regard the degree of poisoning

<table>
<thead>
<tr>
<th>Degree of poisoning</th>
<th>Poisoned patients (n)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>15</td>
<td>25.0</td>
</tr>
<tr>
<td>Moderate</td>
<td>39</td>
<td>65.0</td>
</tr>
<tr>
<td>Severe</td>
<td>6</td>
<td>10.0</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table (3) showed that there was a negative correlation between the severity of poisoning and each of PChE levels and AChE activity %. The cholinesterase enzymes showed highly significant decrease (P<0.001) in severe poisoning compared to moderate and mild poisoning.

Figure 6
Table 3: Comparison between the degree of poisoning and mean values (U/l) and activity percentage of cholinesterase enzymes.

<table>
<thead>
<tr>
<th>Cholinesterase Enzymes</th>
<th>Mild poisoning (100)</th>
<th>Moderate poisoning (500-1000)</th>
<th>Severe poisoning (&gt;500)</th>
<th>P&lt; value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PChE</td>
<td>1.301 ±0.534</td>
<td>0.52 ±0.51</td>
<td>0.25 ±0.22</td>
<td>P&lt;0.001 ***</td>
</tr>
<tr>
<td>AChE</td>
<td>26.36%</td>
<td>20.36%</td>
<td>21.24%</td>
<td>P&lt;0.001 ***</td>
</tr>
</tbody>
</table>

DISCUSSION

The present work was carried out to investigate the relationship between cholinesterase inhibitor insecticides in human blood and insecticides residues in drinking water, in Mansoura city- Egypt. The analyzed drinking water and blood samples by GC-MSD revealed the presence of organophosphorous (Mevinphos) (fig.1) and organochlorine (Aldrin) insecticides (fig.2). However, this result does not necessary indicate that, the drinking water was the source of insecticides poisoning in poisoned patients, as pesticides are used widely for agriculture, vector control, and domestic purposes (7). This fact was proved in our study by the different route and mode of exposure to insecticides among the poisoned patients including accidental and suicidal poisoning, and the clinically variant degree of severity among them, that was confirmed by cholinesterase enzymes assay.
As regard age, the study showed that (46.67%) were in the age group from 20 to less than 30 years (table 1), and suicidal mode of poisoning was common among this group particularly in females. This finding is in agreement with the result found by Agarwal (3), who reported a peak incidence of poisoning by cholinesterase inhibitor insecticides in the 3rd decade of life. It is obviously due to the fact that this age group is the determining factor of the life in terms of studies, service, marriage and other life settlement factors. Therefore, they are subjected to substantial amount of mental stress and strain during this period. Moreover, 43.33% of patients were in age group from 10 to less than 20 years, this correlates with age ratio reported by Marey (13).

There was a female sex predominance among the poisoned patients (56.67%) (fig.3). Female preponderance in this study could be accounted to the fact that females are more often exposed to the stress and strain of day to day life, as well as to the occupational hazards than the males in this place.

Compared to mild and severe poisoning, moderate poisoning of cholinesterase inhibitor insecticides (according to cholinesterase enzyme levels) was the commonest among the studied group (65.0%) (table 2). There was a negative correlation between the severity of poisoning and each of PChE levels and AChE activity %, being lowest in severe poisoning compared to moderate and mild poisoning (table 3) as proved by (2,12).

Presence of Aldrin in drinking water is attributed to contamination from industrial effluents and soil erosion during irrigation, where River sediments may contain higher amounts (up to 1 mg/Kg) (19). Existence of Aldrin in the investigated drinking water samples represents a serious environmental problem for human. According to WHO (20) and EMOHR (8), Aldrin is very dangerous organic pollutant in surface water where the target organs being the central nervous system and the liver. It has been regulated as a possible human carcinogen primarily on the basis of animal studies, however, the epidemiologic evidence is inconsistent (15). On the other hand, this compound induced immunosuppressive and hormonal disruption activities in animals and humans (16,5).

**CONCLUSION AND RECOMMENDATIONS**

Drinking surface water in the area of study was polluted with organophosphorous and organochlorine insecticides compounds. The blood of patients complaining of cholinesterase inhibitor insecticides poisoning showed the same insecticides which were detected in the drinking water. Presence of these pollutants in surface drinking water of the studied area has serious effects on human health and environment.

The following recommendations would be considered by local authority in Dakahlia governorate:

- Multiple-stage filters containing granular activated carbon as an adsorbent material (with replaceable cartridges) could be used mainly in drinking water stations and in houses.

- Chemical analyses could be carried out periodically for the surface and groundwater to ensure the water suitability for drinking purposes (water must be free from pesticide residues or within the permissible limit of WHO).

- Complete knowledge of organophosphorous and organochlorine insecticides residues and their transformation products in surface water is one of the most important factors for solving the environmental problem related to these chemicals.

- Authorities must prohibit the use of organochlorine and restrict the use of organophosphorous insecticides.

**References**

13. Marey, H.M. (1986): Toxicological study on cases of organophosphorous insecticides admitted to the emergency department of Alex. Main university hospital. MSc emergency medicine, Faculty of medicine; Alex. University.
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