Evaluation of Adverse Health Effects of Pesticides Exposure [Biochemical & Hormonal] among Egyptian Farmers

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ABSTRACT

Objective: the aim of this study was to evaluate impact of pesticides on Egyptian farmers health by studying some biochemical (liver and renal functions) and hormonal (testosterone, thyroid hormones; triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) parameters following the exposure of farmers to different periods to pesticides. In addition, clarify some underlying mechanisms of organophosphates toxicity through measuring total antioxidant capacity, lipid per oxidation markers (Malondialdehyde [MDA]) and hematological profile. Subjects and Methods: Forty five male Egyptian farmers who deal with pesticide handling and spraying were recruited in this study together with fifty control subjects. Testosterone, thyroid hormones; triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH) parameters were estimated together with liver and renal functions, determination of serum malondialdehyde and hematological profile was done. Results: As regards the hormonal changes; the exposed group had significantly lower levels of testosterone, T3 and T4, compared to the control group, while TSH was significantly higher in exposed farmers compared to controls, while, the blood MDA level was significantly higher in the exposed farmers than the controls. The hematological parameters showed significant increase in WBC count in the pesticide-exposed group. Enzyme activities of AST and ALT were significantly raised among the exposed farmers, while serum levels of urea and creatinine in the exposed group showed insignificant elevation as compared to controls. Conclusion: It can be concluded that chronic exposure to organophosphate can cause endocrine disrupting effects, liver and renal function impairment and oxidative stress. Further researches on large samples are needed to confirm these findings and to obtain detailed information about mechanisms of toxicity. Also, official regulations and interventions to reduce farmers' overexposure to pesticides are also needed.

Key words: pesticide hazards, Liver & renal functions, T3, T4, TSH, Testosterone, MDA

Introduction

Pesticides are increasingly used worldwide to enable increase production of higher quality crops and overcome of some diseases. The severity of pesticide hazards is much pronounced in third world countries. A number of long persistent organochlorines, which have been severely restricted are still marketed and used in many developing countries. Acute poisoning with organophosphate (OP) in human is frequently seen in many countries and it is estimated to be the cause of more than 200,000 deaths around the world (Attia, 2006).

Occupational exposures to these pesticides occur from skin absorption and inhalation, and the toxicity may be attributed to a number of reasons, including farmers’ poor knowledge about pesticides and pesticide use, less protection against exposures, minimal understanding of the health risks and, most importantly, inadequate safety warnings on the packages provided by the manufacturers (Gbaruko et al., 2009).

It has been reported that, pesticides have been shown to cause over production of reactive oxygen species (ROS) in both intra- and extracellular spaces, resulting in a decline of sperm count and infertility(Aaf et al.,2009). ROS has been shown to damage macromolecules, including membrane bound polyunsaturated fatty acid (PUFA), causing impairment of cellular function(Lenzi, 2000).

The reactive oxygen species (ROS) have been implicated in the toxicity of the pesticides. Organophosphates induce characteristic changes of oxidative stress (Abdollahi et al., 2004).A significantly elevated MDA (end product of lipid peroxidation) level was observed in the sprayers exposed to organophosphate, carbamate, and organochlorine pesticides, when compared to the controls (Prakasam et al., 2001), suggesting that oxidative stress may be involved in the toxicity of pesticides (Hai et al., 1997).

Pesticides differ greatly in their mode of action, uptake by the body, metabolism and toxicity to humans(Anwar, 1997). Pesticide exposure may produce biochemical changes even before adverse clinical
health effects are manifested in these farmers. Prolonged exposures to pesticide affect multiple organs including liver and kidney which can be detected by serum enzymes and other biochemical parameters among farm workers (Hernandez et al., 2006).

Zaidi study showed that the level of TSH was elevated also in male pesticide formulators exposed to the dust and liquid formulation of endosulfan, quinalphos, chlorpyriphos, monocrotophos, lindane, parathion, phorate, and fenvalerate as compared to a control group, but the increase was statistically insignificant (Zaidi et al., 2000). Occupational exposure to pesticides causes many adverse health effects and several studies revealed that farmer exposed to pesticides for prolonged periods are more likely to develop reproductive, developmental, immunological effects, and prostate cancers than the general population (Yassin et al., 2002). Chronic exposures to pesticides also produce changes in body parameters and cause liver and kidney dysfunction. Moreover, certain neurologic pathologies (Elbaz et al., 2009), respiratory symptoms (Salameh et al., 2003) and hormonal and reproductive abnormalities have also been associated to pesticide exposure. However, the disorders generated by the endocrine disruptive pesticides can be temporary or permanent (Garry et al., 2003).

The excessive use of these pesticides with the absence of basic security measures makes Egyptian farmers the most exposed ones. The aim of this study was to evaluate impact of pesticides on some biochemical (liver and renal functions) and hormonal (testosterone, thyroid hormones; triiodothyronine (T3), thyroxine (T4) and thyroid stimulating hormone (TSH)) parameters following the exposure of farmers to different periods to pesticides. In addition, clarify some underlying mechanisms of organophosphates toxicity through measuring total antioxidant capacity, lipid peroxidation markers (Malondialdehyde) and hematological profile.

**Subjects and methods:**

Fifty five male Egyptian farmers who deal with pesticide handling and spraying from Minoufiya Governorate were recruited in the present study, besides fifty control subjects' residents of the same area were included in this study. Informed consents were obtained from all subjects. The potentially pesticide exposed farmers were randomly selected from the farms on the basis of their full time active involvement in preparation, storage and spraying of the pesticides mainly organophosphates on the crop. The pesticide exposed farmer and control group were matched as regard gender, residence, socioeconomic standard, marital status, smoking habit and duration of work. Their age ranged from (28-47 years). The exposure period of farmers to different pesticides were found to be between 2 and 9 years.

Medical history and physical examination of the subjects were carried out before the start of the study. The farmers suffering from diabetes mellitus, hypertension, chronic renal failure, viral hepatitis or any other chronic illness not related to pesticide exposure were excluded from the study.

Blood samples: 5 ml of blood was collected into a sterile labeled tube and allowed to clot, and then the blood was centrifuged at 3000 rpm for 10 minutes. The serum was removed and stored at -20 °C until assay of biochemical and hormone parameters.

**Laboratory investigations:**

1) **Quantitative determination of Testosterone (ng/ml) in human serum by enzyme immunoassay using commercially available kit DRG® Testosterone ELISA (EIA-1559), the DRG® testosterone ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA), based on the principle of competitive binding (Tietz, 1986).**

2) **Estimation of thyroid stimulating hormone (TSHµlu/ml) in human serum by Enzyme Immunoassay (EIA-Quantitative determination of TSH 1782) from DRG International Inc., USA. The DRG TSH ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the intact TSH molecule (Engall, 1980).**

3) **Quantitative Determination of total triiodothyronine (Total T3ng/dL) in human serum by ELISA kit, Cat. No. 1700; from Alpha Diagnostic Intl. Inc. USA. Total T3 ELISA kit is based on competitive binding of human thyroxine from serum samples and enzyme-labeled T3 to T3-specific antibodies immobilized on microtiter well plates (Spector et al., 1976).**

4) **Quantitative Determination of total thyroxine (total T4µg/dL) in human serum by ELISA kit, cat. No. 1100 from Alpha Diagnostic Intl. Inc. USA. Total T4 ELISA kit is based on competitive binding of human thyroxine from serum samples and enzyme-labeled T4 to T4-specific antibodies immobilized on microtiter well plates (Schall et al., 1978).**

5) **Determination of Malondialdehyde (MDAnmol/ml) in serum was measured according to the method (Ohkawa et al., 1979), MDA level was determined by thiobarbituric acid reactive substances (TBARS). The principle of this method was based on the spectrophotometric measurement of the color occurring during the reaction to thiobarbituric acid with MDA. Concentration of thiobarbituric acid reactive substances was calculated by the absorbance coefficient of malondialdehyde-thiobarbituric acid complex.**

6) **Liver function tests:**
Determination of serum aspartate transaminase (AST) and serum alanine transaminase (ALT) by using kinetic method recommended by the Committee on Enzyme of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974), the test was performed using already commercially available kit from Boehringer-Mannheim Company, Germany.

7) Kidney function tests:
   a- Determination of serum creatinine level (mg/dl) according to the Jaffe reaction (Husdan and Rapoport, 1968).
   b- Determination of serum urea (mg/dl) level was done by using chemical colorimetric method QuantiChrom™ Urea Assay Kit (DIUR-500). Bioassay Systems’ urea assay kit is designed to measure urea directly in biological samples without any pretreatment. The improved Jung method utilizes a chromogenic reagent that forms a colored complex specifically with urea. The intensity of the color, measured at 520nm, is directly proportional to the urea concentration in the sample (Jung et al., 1975).

8) Hemogram: including total red blood cell (RBC) count (x10^6 mm^-3), hemoglobin content (Hb) (g /dL), total number of leukocytes (x10^3 mm^-3) and Platelet count (x10^3 mm^-3) were assessed using Coulter counter and examination of Leshman or Wright-stained peripheral blood smears.

Statistical Analysis:

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 11(SPSS Inc., Chicago, USA). Standard descriptive statistics were used to summarize the hormonal levels and biochemical parameters such as means and standard deviations (SD). Significant differences between mean values of exposed and control groups were statistically analyzed using the Student's t-test. Results were considered significant when p-value < 0.05.

Results:

The obtained results show no statistically significant differences between the exposed farmers and the control groups as regard gender, age, socioeconomic status and tobacco smoking (P>0.05); except years of exposure to pesticides which was highly significant among the exposed farmers (P<0.05) (Table 1).

Table (2) showed that the enzyme activities of AST and ALT, were significantly raised (P<0.05) among the pesticide exposed farmers as compared to control group. Serum levels of urea and creatinine in the exposed group showed insignificant elevation (P>0.05) as compared to controls but it was of clinical value that denoting of slight or early renal impairment.

As regards the hormonal changes table (3) demonstrates that the exposed group had significantly lower levels of testosterone, T3 and T4, compared to the control group (P<0.05), while TSH was significantly higher in exposed farmers compared to controls (P<0.05). While the blood MDA level, as an end product of lipid peroxidation, was significantly higher in the exposed farmers than those in the controls (P<0.05).

No significant differences were found between most of the hematological parameters in pesticides-exposed farmers and the control groups (table4), however, there is significant increase in WBC count (P<0.05) observed in the pesticide-exposed group as compared to the control group.

Table 1:

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exposed farmers</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>50</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>37.9±5.7</td>
<td>37.5±5.1</td>
<td>0.45</td>
</tr>
<tr>
<td>Tobacco smoking (no. of cases)</td>
<td>13 (26 %)</td>
<td>15 (27.27 %)</td>
<td>0.72</td>
</tr>
<tr>
<td>Experience of pesticide exposure (year)</td>
<td>0</td>
<td>8.5±2.1</td>
<td>0.001*</td>
</tr>
<tr>
<td>Socio-economic status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Middle</td>
<td>30 (60%)</td>
<td>29 (52.7%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Low</td>
<td>20 (40%)</td>
<td>24 (43.6%)</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD
* indicates significance (P < 0.05)

Table 2:

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Exposed farmers</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>50</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>ALT (U/L) Mean ± SD</td>
<td>29.9±2.7</td>
<td>45.5±2.9</td>
<td>0.001*</td>
</tr>
<tr>
<td>AST (U/L) Mean ± SD</td>
<td>22.9±2.0</td>
<td>38.0±2.0</td>
<td>0.005*</td>
</tr>
<tr>
<td>Urea (mg/dL) Mean ± SD</td>
<td>16.8±2.6</td>
<td>19.5±1.8</td>
<td>0.092</td>
</tr>
<tr>
<td>Creatinine (mg/dL) Mean ± SD</td>
<td>0.85±0.05</td>
<td>1.2 ±0.4</td>
<td>0.077</td>
</tr>
</tbody>
</table>

ALT= Alanine Aminotransferase, AST= Aspartate aminotransferase
* indicates significance (P < 0.05)
**Table 3: Comparison of some hormonal levels and MDA in the studied groups.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Exposed farmers</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone (ng/ml)</td>
<td>Mean ± SD</td>
<td>26.4±2.1</td>
<td>17.7±4.4</td>
</tr>
<tr>
<td>TSH (µg/ml)</td>
<td>Mean ± SD</td>
<td>2.6±1.0</td>
<td>5.4±1.3</td>
</tr>
<tr>
<td>T_3 (ng/dL)</td>
<td>Mean ± SD</td>
<td>11.8±0.2</td>
<td>8.0±1.9</td>
</tr>
<tr>
<td>T_4 (ng/dL)</td>
<td>Mean ± SD</td>
<td>167.4±10.3</td>
<td>142.9±9.8</td>
</tr>
<tr>
<td>MDA (mmol/mL)</td>
<td>Mean ± SD</td>
<td>3.2±0.6</td>
<td>6.8±1.6</td>
</tr>
</tbody>
</table>

TSH= Thyroid-stimulating hormone, T_3= Thyroxine, T_4= Triiodothyronine
MDA= Malondialdehyde
* indicates significance (P < 0.05)

**Table 4: Hematological profile in studied groups (Values are expressed as mean ± SD).**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Exposed farmers</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs(x10^6 mm^-3)</td>
<td>5.1±0.2</td>
<td>5.5±0.6</td>
<td>0.5561</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>14.4±0.5</td>
<td>14.9±0.7</td>
<td>0.6215</td>
</tr>
<tr>
<td>WBCs(x10^3 mm^-3)</td>
<td>12.7±1.6</td>
<td>8.5±0.5</td>
<td>0.01275*</td>
</tr>
<tr>
<td>PLT(x10^3 mm^-3)</td>
<td>272.2±55.03</td>
<td>258.9±26.08</td>
<td>0.5832</td>
</tr>
</tbody>
</table>

RBCs= Red Blood Cells, Hb=Haemoglobin
WBCs= White Blood Cells, PLT= Platelets
* indicates significant (P < 0.05)

**Discussion:**

Pesticides are "poisons by design" and poisonings resulting from unsafe use of these chemicals are the most prevalent and serious occupational hazards faced by agricultural workers in developing countries. Organophosphate (OP) pesticides are among the leading chemicals used extensively for agricultural pests control throughout the world. Use of pesticides not only affects our environment but also affects the health of the farmers (Ibitayo, 2006).

In this work, the exposed farmers had a significantly lower triiodothyronine (T_3) and thyroxine (T_4) serum levels as well as a significantly higher thyroid stimulating hormone (TSH) when compared to control group. These results were in accordance with Zaidi et al., 2000 who stated that assessing formulators of both organochlorines, specifically lindane, and organophosphate insecticides showed an increase in TSH levels and a decrease in T_3 levels in workers compared with controls, therefore, pesticides may also affect the binding of thyroid hormones to thyroid receptors, resulting in an alteration in the expression of genes. This may then affect thyroid hormone metabolism and thereafter alters their levels. Furthermore, pesticides may activate the nuclear receptor constitutive androstane receptor, which in turn induces enzymes involved in thyroid hormone metabolism, including uridine 5’-diphosphate-glucuronosyltransferase and sulfotransferase. This may leads to increased hepatic metabolism of thyroid hormones, resulting in decreased plasma T_3 and increased TSH level.

The results of our study showed that the exposed group had significantly lower values of testosterone compared to the control group (p<0.05) this is in accordance with Sahar et al., 2011 who demonstrated that serum testosterone levels is significantly lower in pyrethroid exposed workers compared to the control group. Moreover, Meeker study has found inverse associations between pesticides and malle testosterone levels. The study found that high levels of the urinarymetabolites of chlorpyrifos (TCPY) and carbaryl and naphthalene(1N) correlate directly with low levels of testosterone in male subjects (Meeker, 2006). Also, in Ray and his colleagues (1991)study showed that quinalphos may exert a suppressive effect on the functional activity of accessory sex glands by decreasing testicular testosterone production following inhibition of pituitary gonadotrophins release. In another study it had been shown that there were lower circulating total and free testosterone concentrations and higher serum gonadotropins (FSH and LH) levels in the Parathion/Paraaxon sprayers than the corresponding reference values (Sherif et al., 2012). Soliman et al 2008 revealed decrease in serum total testosterone levels among men exposed to pesticides or solvents in Damietta Governorate. On the other hand, Kamijima et al 2004 found a significantly increased concentration than normal of serum testosterone concentrations after organo- phosphorous exposure during the off-season though FSH and LH concentrations were normal during both busy and off seasons.

The enzyme activity of AST and ALT were significantly raised among the pesticide exposed farmers as compared to control group. These findings coincide with Al-sarar et al., 2009 who reported a slight elevation in AST, ALT and ALP serum levels in pesticides-exposed workers of Riyadh municipality, Saudi Arabia. Significant increase in the levels of these enzymes, which was positively correlated with pesticide residues, was found in occupationally exposed tobacco farmers in Pakistan (Khan et al., 2008).

In our study, serum urea and creatinine showed statistical insignificant elevated levels but clinical has an impact of slight or beginning of renal impairment as compared to controls. This is in parallel with Al-Qarawi and Adam...
(2003) who revealed insignificant elevation of serum urea and creatinine levels among pesticide sprayers. Previous study covered workers of a chemical plant producing dust pesticides reported minimal nephrototoxic changes in these workers with higher levels of serum creatinine and urea. A subtle nephrototoxic changes in workers occupationally exposed to pesticides was reported, because of their higher levels of serum creatinine and/or blood urea (Hernandez et al., 2006). In contrast to these results Dilshad et al., study revealed significantly elevated levels of serum urea & creatinine among exposed farmers.

Pesticide-induced oxidative stress has been the focus of toxicological research for over a decade as a possible mechanism of toxicity. Toxic effects of pesticide on human beings specially by omitting radical production can be confirmed by the direct measurement of lipid peroxidation by-product Malondialdehyde (MDA) (Muniz et al., 2007). Our study showed that the blood MDA level was significantly higher in the exposed farmers than those in the controls. Our results were consistent with other study that suggested that pesticides increase oxidative stress in humans which showed that the Malondialdehyde, the last product of lipid peroxidation was found to be increased significantly in sprayers as compared to the controls, moreover serum MDA levels was 4.9 times and 24 times higher in farm workers and applicators respectively than in controls (Singh et al., 2007).

As regard hematological profile, our results showed no significant differences between hematological parameters in pesticides-exposed and control groups except in WBC count which was significantly increased in pesticides-exposed group. Our result supports the earlier findings of Al-Sarar et al., (2009) who found no significant differences between hematological parameters in pesticides-exposed and control groups. However, a significant increase in WBC count (p<0.01) was observed in the pesticide-exposed group and significant increases in lymphocytes and monocytes counts were recorded in this group.

It was reported that a decrease in the mean corpuscular haemoglobin concentration (MCHC) and in the mean platelet volume (MPV) were 38% and15% respectively of greenhouse sprayers chronically exposed to pesticides (Hernandez-Valeno et al., 2000) The main alterations found in the total and differential white blood cell count were the increase of monocytes in 5% of workers and of eosinophil in 4% of these sprayers. Studies on human toxicity of pesticides had also focused on biological parameters related to organ functions.

Conclusion:

It can be concluded that chronic exposure to organophosphate can cause endocrine disrupting effects, liver and renal function impairment. Also, increased level of serum MDA in exposed farmers is probably reflective of increased lipid per oxidation and cell damage (Oxidative stress). So further researches on large scales are needed to confirm these findings and to obtain detailed information about mechanisms of toxicity. Also, official regulations and interventions to reduce farmers' overexposure to pesticides are needed.

References


