

Clinical and Biochemical Parameters of Children and Adolescents Applying Pesticides

¹Community, Environmental and Occupational Medicine Department, Faculty of Medicine, Menoufia University, Shebin Elkom, Egypt.

²Center for Research on Occupational and Environmental Toxicology (CROET), Oregon Health and Sciences University (OHSU), Portland, Oregon, USA.

³Clinical Pathology Department, National Liver Institute, Menoufia University, Shebin Elkom, Egypt.



AA Ismail,¹ DS Rohlman,² GM Abdel Rasoul,¹ ME Abou Salem,¹ OM Hendy³

Abstract

Background: The primary agricultural product in Egypt is the cotton crop. Children and adolescents work seasonally in the cotton fields applying pesticides.

Objectives: To examine the effect of pesticide exposure on clinical and biochemical parameters in children and adolescents applying pesticides.

Methods: Male children currently applying pesticides and aged between 9 and 19 years (n = 50) were recruited for this study. They were asked to complete work, health, and exposure questionnaires; examined for any medical and neurological problems with particular attention to sensory and motor functions including cranial nerves, sensory and motor system, and reflexes. From each participant, a blood sample was taken to measure acetylcholinesterase activity, and liver and kidney functions. Children who have never worked in agriculture (n = 50), matched on age, education, and socioeconomic status were also studied and served as controls.

Results: More neuromuscular disorders were identified in pesticide applicators than controls. A significant lower level of acetylcholinesterase was found in the applicator group compared to the controls. There was also a significant difference in hematological, renal and hepatic indices in the exposed children compared to the control children. Working more days in the current season and also working more years as a pesticide applicator were both associated with an increase in the prevalence of neuromuscular abnormalities and significant changes in the laboratory tests.

Conclusion: Children and adolescent pesticide applicators working in farms of Egypt are at risk of developing serious health problems similar to those of adults.

Keywords: Neurologic manifestations; Adolescent; Pesticides; Biochemical processes; Seasonal applicators; Acetylcholinesterase

Introduction

The primary agricultural product in Egypt is the cotton crop and nearly 40% of the Egyptian workforce is employed in agriculture.¹ Pesticides are regularly used in this country on cotton and other crops and their use is regulated by

the Ministry of Agriculture.² Children and adolescents work seasonally in the cotton fields applying pesticides.

The health effects of pesticide exposure on adult pesticide applicators and farm workers who use pesticides have been shown in many studies. A review of these

Correspondence to
Ahmed Ismail, MD,
Public Health and
Community Medicine
Department,
Faculty of Medicine,
Menoufia University,
Shebin Elkom,
Egypt
E-mail: aa-ismail@
hotmail.com

studies demonstrated a wide range of neuromuscular disorders, acetylcholinesterase (AChE) inhibition and deterioration of the hematological, hepatic and renal functions associated with exposure to pesticides.³⁻⁵

Previous research studies with pesticide applicators have revealed a wide range of neuromuscular disorders that affect the sensory and motor functions. Fifteen out of 19 studies showed a variety of abnormalities in the neuromuscular system in applicators compared to controls. The reported disorders included nerve function abnormalities,⁴ paresthesia,⁶⁻⁸ increased sensitivity to vibration,⁹ disturbed balance,⁵ tremors,⁷ staggering and weakness,⁴ hyper-reflexia,⁸ and loss of muscle strength in legs or arms and difficulty in moving fingers or grasping things.¹⁰ Kamel, *et al*,³ reported most of these disorders in a cohort of licensed private pesticide applicators. However, other researchers reported no significant difference in the prevalence of neuromuscular disorders between farm workers who apply pesticides and control groups.^{11,12}

Nine studies investigated the effects of pesticide exposure on the level of AChE in pesticide applicators. Three studies measured the AChE level before and after exposure to pesticide, mainly organophosphorus, and found a significant drop in its level after the exposure.¹³⁻¹⁵ The other studies measured AChE level only at one time. However, these studies also revealed that the level of AChE was significantly lower in those who had exposure to pesticide than non-exposed groups.^{6,8,16-19}

Hematologic profile, hepatic and renal functions were used to assess the effects of occupational exposure to pesticide on the hematopoiesis and function of the liver and of the kidneys among pesticide applicators. Patil and his colleagues,¹⁸ found significantly lower serum levels of total

proteins and albumin, as well as lower hematological parameters, *i.e.*, hemoglobin (Hb), hematocrit and red cell (RBC) indices. They also reported a significant increase in lipid peroxidation, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) in pesticide applicators compared to a control group who had not been exposed to any kind of pesticide. Srivastava and his coworkers,⁸ found significantly higher levels of serum alkaline phosphatase (ALP) in those occupationally exposed to quinalphos in comparison with controls. Other studies found that mean corpuscular hemoglobin (MCH) was significantly lower in those with high exposure to pesticides than people with low exposure to pesticides.⁷ Moreover, ALP and glutamate oxaloacetate transaminase (GOT) levels were significantly higher in pesticide sprayers than in the controls.²⁰ White blood cell (WBC) and platelet counts were increased and Hb was decreased significantly in the exposed farm workers at the end of the spraying day compared to counts at the start of the day.¹³

Only two studies have examined the sensory and motor functions and biochemical parameters in those with exposure to pesticides in Egypt. When Egyptian pesticide applicators working in cotton crops were compared with control participants who were never occupationally exposed to pesticides, they reported a significantly higher prevalence of symptoms including blurred vision, dizziness, numbness and/or tingling, paresthesia, headache, vertigo, asthenia, as well as signs of superficial sensory loss, diminished or lost ankle and deep tendon reflexes, and trophic and vasomotor changes.² Another study reported that pesticide workers complained more often of symptoms like dizziness and numbness in a neurological exam than a control group who had never been ex-

TAKE-HOME MESSAGE

- Children and adolescents may work as seasonal pesticide applicators in cotton fields in Egypt.
- Occupational exposure to pesticide has several clinical and biochemical implications including neurobehavioral deficits, neuromuscular disorders, lower levels of serum acetylcholinesterase, and significant changes in many laboratory parameters.
- As duration of work increases, we witness a higher prevalence of neuromuscular signs and laboratory abnormal results.

posed to pesticides.¹⁷

The significant anatomical and maturational physiological differences of children and adolescents with adults make them more vulnerable to pesticide exposure, hence the recent concerns about the impact of pesticides on children and adolescents.²¹ As an example, the neurodevelopmental effects of chlorpyrifos pesticide have been demonstrated in animal studies.²² Although several studies have examined children who live in agricultural communities or whose parents work in agriculture, there are no studies that have examined the effects of exposure to pesticides on clinical and biochemical parameters of adolescents applying pesticides.

A previous report from our group examined the neurobehavioral deficits, neurological symptoms, and personality changes in adolescent pesticide applicators compared to controls and the relationship between their neurobehavioral deficits and their AChE levels and duration of work.²³ We conducted the current study to examine the neuromuscular signs

and any changes in clinical and biochemical parameters in children and adolescents exposed to pesticides and the relationship between these effects and AChE levels and work experience.

Patients and Methods**Participants**

This study was conducted from June to August 2005, during the pesticide application period for the cotton crop. It was carried out in the Shebin El Kom District, Menoufia governorate, Egypt. During this period, the local agricultural office hired approximately 10 children from each village to apply pesticide to the cotton crop under the supervision of adult engineers and agricultural employees. We used a random cluster sampling; five villages out of 50 from the Shebin El Kom District were randomly selected to recruit children and adolescents working as pesticide applicators as study participants. Male children aged between 9 and 18 years were included in this cross-sectional study. There were a total of 56 children working in these five villages. Six children declined to participate which translated to a response rate of 89%.

The control group was composed of children who had never worked in the cotton fields and had similar age, socioeconomic status, and educational level as the applicator children. Fifty control children were randomly selected from the friends and relatives of the applicator children. The control children lived in the same community as the children applying pesticide and attended the same schools.

Pesticide application

The procedure was explained in detail elsewhere.²³ The pesticide applications for the cotton crop are highly regulated in Egypt and the Ministry of Agriculture has spe-

cific guidelines that are followed throughout the country. During 2005, pesticides were applied four times between June and August. In the first cycle, *Bacillus thuringiensis*—a natural occurring bacterium that is harmful to insects but not humans—are used; the second cycle includes application of Pestban (chlorpyrifos); in the third cycle, Pyrethrins or a less potent carboxylate is applied; in the fourth cycle, Dursban (another formulation of chlorpyrifos) is used. There is approximately a week of no spraying between the four application periods. This schedule of pest management has been followed rigidly for the past 10 years. The typical workday was from 8:00 to 12:00, followed by 15:00 to 19:00, six days per week. Organophosphate pesticides were applied during the second and fourth cycles, from the end of June through the first week of July and during the second week of August.

Pesticides were applied by Agremond backpack sprayers which can hold 20 L of pesticide solution. At each location, spraying was carried out by a team of seasonal workers that consisted of 7–12 applicators. There are no regulations in Egypt requiring personal protective equipment and there is no formal training on its use. Thereby, personal protective equipment is not commonly used by the applicators. Therefore, the main routes of exposure are through inhalation and dermal exposure.

Procedures

The participants were examined at the end of the workday in a clinic near their resident villages during the last week of the spraying season. Informed written consent was obtained from the children and their legal guardians. The study was approved by the Ethics Committee of the Faculty of Medicine, Menoufia University on July 13, 2005.

The participant children, with the as-

sistance of their parents, completed a questionnaire containing questions about medical and work history with information about their exposure to pesticides. A clinical examination was done consisting of general, chest, heart, and abdominal exams, as well as specific neurological tests for assessing sensory and motor functions including: 1) cranial nerves (sense of smell, visual acuity, visual fields, pupillary reactions, extraocular muscle movements, corneal reflexes, hearing and gag reflex); 2) motor system (involuntary movements, muscle status, muscle tone, muscle power, and coordination); 3) reflexes (knee and ankle and plantar reflexes); and 4) sensory system (superficial sensations—pain and touch sensation; deep sensations—sense of vibration; position and discriminate sensations—stereognosis, number identification, and two-point discrimination). The examiner was masked to the group the child belonged to—pesticide applicators or control group. Blood sample was drawn from all participants and serum AChE was measured according to Weber,²⁴ using standard laboratory kits (Test-combination Boehringer Mannheim GmbH Diagnostica). Complete blood count, liver and kidney function tests were also done using the methods described by Tietz.²⁵

Statistical analysis

SPSS version 14.0 (SPSS Inc, Chicago, IL, USA) was used to analyze the data. Normally distributed continuous variables are presented as mean±SD. Non-normally distributed continuous variables are presented as median and interquartile range (IQR). *Student's t* test was used to examine the difference between means in case of the normally distributed variables, while Mann-Whitney U test was used in case of non-normally distributed variables; χ^2 was used to examine the difference between percentages; the Pearson's

correlation coefficient was used to examine the correlation between two continuous normally distributed variables, while its equivalent, the Spearman's rho correlation coefficient was used for assessing correlation between two continuous non-normally distributed variables. We also used "effect size" as a measure of the relationship between two variables. It is often useful to know not only whether an experiment has a statistically significant effect, but also the size of any observed effects. We calculated the "effect size" according to Cohen,²⁶ as $(M_1 - M_2)/SD$, where M_1 and M_2 represent means of the two groups and SD is the pooled standard deviation.

Results

Demographic characteristics

Demographic characteristics of the participants showed no significant difference between the exposed (14.38 ± 2.45 , 6.20 ± 4.06 , and 54%, respectively) and the control (14.21 ± 2.67 , 6.90 ± 3.85 , and 48%, respectively) groups in terms of mean \pm SD

age, mean \pm SD years of education, or the prevalence of smoking habits.

Duration of work and AChE

The exposed children reported that they had worked as pesticide applicators for a mean duration of five years (range: 1 to 9). During the current application season, they reported working between 3 and 30 days, prior to testing (mean: 20 days). AChE was measured for participants in both exposed and control groups. The mean \pm SD AChE activity was significantly ($p < 0.001$) lower in pesticide applicators (239.8 ± 60.0 IU/L) than in the control group (283.1 ± 61.6 IU/L).

Neuromuscular signs

The neurological examination revealed a significantly higher ($p < 0.05$) prevalence of neuromuscular signs in the exposed participants than the control group. Pesticide applicators had more abnormalities in the knee reflex, superficial sensation and coordination ($p < 0.05$; Odd's ratio ranged from 3.9 to 7.6) (Table 1).

Table 1: Comparison of the number of pesticide applicators (exposed) and the control participants with neuromuscular signs.

Neuromuscular Signs	n (%)		Odd's ratio (95% CI)
	Exposed (n=50)	Control (n=50)	
Abnormalities in:			
Movements (tremors)	8 (16)	2 (4)	4.57 (0.92–22.74)
Muscle power	5 (10)	1 (2)	5.44 (0.61–48.42)
Ankle reflex	6 (12)	1 (2)	6.68 (0.77–57.73)
Knee reflex	10 (20)	2 (4)	6.00 (1.24–29.00)
Superficial sensation	12 (24)	2 (4)	7.58 (1.60–35.95)
Deep sensation	1 (2)	0 (0)	—
Coordination	10 (20)	3 (6)	3.92 (1.01–15.23)

Table 2: Laboratory findings in pesticide applicators (exposed) and the control group.

Parameter	Mean±SD		Effect size	p value
	Exposed (n=50)	Control (n=50)		
RBC count (10 ⁶ /μL)	4.1±0.2	4.8±0.6	-1.6	<0.001
Hemoglobin (g/dL)	12.4±1.2	13.3±1.0	-0.8	<0.001
Leukocytes (10 ³ /μL)	6.5±0.3	7.2±0.4	-2.0	<0.001
Lymphocytes (10 ³ /dL)	2.4±0.8	3.0±0.9	-0.7	<0.001
Basophils (10 ³ /dL)	79.1±34.0	76.8±23.6	0.1	0.46*
AST (IU/L)	29.7±2.1	28.4±2.7	0.5	0.009
ALT (IU/L)	32.8±3.0	31.3±3.6	0.5	0.026
ALP (IU/L)	170.0±58.0	154.7±57.0	0.3	0.1*
Total protein (g/L)	6.2±0.9	7.5±1.0	-1.4	<0.001
Albumin (g/L)	3.5±0.4	4.1±0.5	-1.3	<0.001
Globulin (g/L)	2.3±0.5	1.9±0.6	0.7	<0.001
A/G ratio	1.6±0.4	2.5±0.9	-1.3	<0.001
Blood Urea Nitrogen (mg/dL)	27.8±5.0	25.4±4.4	0.5	0.012
Creatinine (mg/dL)	1.1±0.2	1.0±0.3	0.4	0.053

*Mann-Whitney U test

Laboratory investigations

Most of the measured hematologic, hepatic and renal function indices were significantly different between the pesticide applicators and control groups. RBC count, Hb, and WBC count were significantly ($p<0.001$) lower in the exposed than in the control group (Table 2). While serum total protein, albumin levels and albumin/globulin (A/G) ratio were significantly lower, AST, ALT, and globulin levels were significantly higher in the pesticide applicators compared to the control group

($p<0.05$) (Table 2). Blood urea nitrogen (BUN) as an indicator for the kidney function were also significantly higher in the exposed than the control group ($p<0.05$). The absolute effect size of these significant effects ranged from 0.4 to 2.0 (Table 2).

AChE and neuromuscular signs

An examination of the relationship between AChE level and the presence of neurological signs revealed that pesticide applicators who had abnormalities of superficial sensation, knee and ankle

Table 3: Pearson's Correlation coefficient (r) or Spearman's rho between numbers of days worked this season, total years worked and acetylcholinesterase (AChE) level with the laboratory investigations, exposed group only.

Parameter	Days worked	Years worked	AChE
RBCs (10 ⁶ /μL)	-0.2	0.1	0.2
Hemoglobin (g/dL)	-0.2	-0.3*	0.2
Leukocytes (10 ³ /μL)	-0.3	-0.13	0.25
Lymphocytes (10 ³ /dL)	-0.3	-0.1	0.2
Basophils (10 ³ /dL) [†]	-0.4*	-0.1	0.5**
AST (IU/L)	0.2	-0.1	-0.3*
ALT (IU/L)	0.5**	-0.1	-0.4**
ALP (IU/L) [†]	0.4*	0.4*	-0.5**
Total protein (g/L)	-0.1	-0.3*	0.1
Albumin (g/L)	-0.1	-0.3*	0.1
Globulin (g/L)	-0.1	0.1	-0.2*
A/G ratio	0.1	0.1	0.2
Blood Urea Nitrogen (mg/dL)	0.31*	0.05	-0.21
Creatinine (mg/dL)	0.2	0.08	-0.21

*p<0.05 **p<0.001 †Spearman's rho

reflexes and coordination had a significantly (p<0.05) lower mean±SD enzyme activity (197.08±40.92, 204.5±39.47, 198.33±35.02, and 194.0±54.71 IU/L, respectively) than those applicators who did not have (253.28±59.12, 248.62±61.39, 245.45±60.75, and 251.25±56.26 IU/L, respectively). This significant difference was not found with any of these signs in the control group.

Correlation between the duration of work, AChE level and laboratory investigations

There is a significant positive correlation between number of days worked in the present season with blood ALT, ALP and BUN level and a negative correlation

with basophils count (p<0.05). Regarding number of years worked, there is a positive correlation with ALP and a negative correlation with Hb, serum total protein and albumin (p<0.05) (Table 3). AChE level had a negative correlation with serum level of AST, ALT and ALP, and a positive correlation with basophils count (p<0.05) (Table 3).

Duration of work and the neurological signs

Comparing the number of days worked this season and total number of years worked for both applicators who had neuromuscular signs and those who did not, revealed that those children report-

Table 4: Comparison of the number of days worked this season and median (interquartile range [IQR]) of years worked as a pesticide applicator, children who did and did not have neuromuscular signs, exposed group only. Mann-Whitney test was used to compare groups with and without a sign.

Neuromuscular signs		Days worked			Years worked		
		n	Median (IQR)	p value	n	Median (IQR)	p value
Movements (tremors)	yes	8	29.0 (4.5)	<0.001	8	8.0 (2.0)	0.001
	no	42	20.0 (11.75)		42	4.5 (8.0)	
Muscle power	yes	5	30.0 (1.5)	<0.001	5	8.0 (3.5)	0.02
	no	45	20.0 (11.5)		45	5.0 (3.0)	
Ankle reflex	yes	6	29.5 (2.3)	<0.001	6	8.5 (2.3)	0.005
	no	44	20.0 (11.0)		44	5.0 (3.0)	
Knee reflex	yes	10	29.0 (3.3)	<0.001	10	8.0 (3.0)	0.001
	no	40	20.0 (11.3)		40	4.5 (3.0)	
Superficial sensation	yes	12	29.0 (2.8)	<0.001	12	8.0 (5.8)	0.008
	no	38	19.0 (11.0)		38	5.0 (3.0)	
Deep sensation	yes	1	30.0	0.12	1	8.0	0.28
	no	49	22.0 (13.0)		49	5.0 (3.5)	
Coordination	yes	10	29.5 (1.5)	<0.001	10	6.0 (5.3)	0.3
	no	40	20.0 (11.3)		40	5.0 (3.8)	

ing signs had worked significantly more days this season for all individual signs but deep sensation ($p < 0.001$) (Table 4), and worked significantly more years for all signs but deep sensation and coordination ($p < 0.05$) (Table 4).

Discussion

The results reported in this study clearly demonstrated several health impacts on children who worked as pesticide applicators compared to the control children. Children who applied pesticides had a higher frequency of neuromuscular signs especially for superficial sensation, knee reflexes and coordination abnormalities

and had lower AChE levels than children from the same community who did not apply pesticides. Also, the exposed group showed significant differences in most of the laboratory tests of blood, and liver and renal functions. Our study also showed an association between the presence of neurological signs and lower levels of AChE, where lower levels of AChE were significantly associated with abnormalities in ankle and knee reflexes and in coordination. This study also showed a correlation between days worked during the current season and the presence of neuromuscular signs and laboratory findings as well as between total years worked as an ap-

plicator and the presence of neuromuscular signs and laboratory test results. This provides evidence for presence of a dose-response relationship between exposure to pesticides and some clinical signs.

The children in this study had an extended exposure time and a high exposure level that was accompanied by a low percentage of personal protective equipment use—only 8% of the children in the study reported using personal protective equipment. This pattern of exposure made these seasonal applicators present with manifestations that were similar to those seen for people who had experienced pesticide poisoning.²⁷

We found that there was a significantly lower level of AChE in pesticide applicators compared to the control children. AChE is used as a biomarker for exposure to organophosphate pesticides.²⁸ The states of Washington and California in the US require AChE monitoring for pesticide applicators.²⁹ However, because of inter-individual variability, a baseline measure is first taken before the season, and then a second measurement is made for each individual to see if a significant depression in the level of enzyme ensues.³⁰ In spite of this requirement, there are many studies that reported only a single measurement of AChE level and still they showed a consistent significant association between exposure to pesticides and AChE inhibition for farm workers.^{6,8,16-19}

Neuromuscular examination revealed that pesticide applicators had a significantly higher prevalence of abnormalities in knee reflex, superficial sensation, and coordination (Table 1). However, there was no significant difference between the exposed and control groups in terms of tremor, muscle strength, deep sensation, and ankle reflex. This pattern replicates the findings of Amr,² who found that adult pesticide applicators showed a higher fre-

quency of neurological signs of superficial sensory loss, decreased or lost ankle reflexes, trophic and vasomotor changes, and decreased or lost ankle and deep tendon reflexes. These findings are also similar to those obtained by Ciesielski, *et al*,³¹ who reported a significant increase in muscular symptoms in the exposed subjects and also effects reported by Farahat, *et al*,¹⁷ who found that pesticide workers reported significantly higher prevalence of numbness.

Hb, RBC, WBC and basophils counts were used to assess the function of the hematopoietic system.³² Pesticides have been found to affect blood forming organ in rats. Many steps in heme biosynthesis were found to be inhibited by pesticidal residues.³³ Another possible mechanism for these effects is the binding of organophosphorus pesticides to iron, followed by a lack of incorporation of iron into Hb.³⁴ This effect may be so strong that it produces aplastic anemia.³⁵

Serum AST, ALT, and ALP levels as well as total protein, albumin, globulin, and A/G ratio have been used to assess liver function. Pesticides decrease total protein and albumin as a result of a decrease in synthesis of albumin in liver, and an increase in globulins (mostly α -globulin).¹⁸ Studying the effect of pesticide exposure in the adolescent pesticide applicators in this study revealed that most of the hematological, renal and hepatic indices were affected; there were significantly lower measures of RBC and WBC counts, Hb level, serum total protein, albumin and A/G ratio in the exposed than the control group. The exposed children had also significantly higher levels of AST, ALT, ALP, globulin, and BUN than the control group (Table 2). Despite the significant effects of pesticide exposure on those laboratory investigations, its clinical importance is not yet established and need more research.

The previous results were confirmed by the presence of a correlation between the duration of exposure and some of these measures; the number of days worked this season had a positive correlation with the serum levels of ALT, ALP and BUN and a negative correlation with basophils count (Table 3). Number of years participants worked had a positive correlation with ALP level and a negative correlation with serum total protein and albumin (Table 3). These results were in keeping with the findings of Abu Mourad,¹³ who found that Hb and hematocrit were significantly decreased after exposure to pesticides, a phenomenon which may be attributed to microcytosis or impaired heme synthesis in the bone marrow,³⁶ and possibly through the binding of organophosphate pesticides to iron in Hb.³⁴ The decrease in lymphocyte count has also been reported in many studies.^{2,7} In contradiction to these results, however, Abu Mourad,¹³ and Paron,⁷ found that the pesticide applicators had significantly higher WBC counts. Many researchers believe that pesticides can stimulate the immune system to induce more WBC activity. The significant increase in the liver enzymes ALT and AST is in parallel with findings of several studies that reported the deterioration of liver enzymes as a result of exposure to pesticides.^{17,18,37} The deterioration of the renal function indicates the hazardous effects of pesticides on the kidneys. Huang, *et al*,³⁸ found that out of 100 rice farmers they studied, 23 had abnormal levels of BUN, 22 of whom had BUN values exceeding the upper normal limit.

Those pesticide applicators who had abnormalities of superficial sensation, knee and ankle reflexes and coordination had a significantly lower AChE level than those without these signs. This difference was not observed with any of these signs in members of the control group. This

significant association exists in the two measures of exposure—both the acute measure, *i.e.*, number of days worked this summer, and the chronic measure, *i.e.*, number of years worked in applying pesticides (Table 3). Lower levels of AChE had also a significant negative correlation with some laboratory parameters (*i.e.*, AST, ALT, ALP and globulin levels) and a positive correlation with basophils count (Table 3), which reflect impaired hepatic functions in those with lower AChE levels. This association between the neurological signs and low levels of AChE was also observed in another report,⁴ which showed farmers applying pesticides who had low AChE levels had more neurological signs than those who did not apply pesticides. Similar results were also reported by Jors, *et al*,³⁹ among farmers in Bolivia. Similar association of AChE level with laboratory test results was also observed by Srivastava, *et al*.⁸

Those pesticide applicators with neuromuscular signs worked more days in the present season or total years worked (Tables 4). Several of the laboratory parameters measured had also significant correlations with the number of days worked in the present season and the number of years worked. There was a dose-response relationship between the presence of neuromuscular signs and the duration work. Similar observations were made by other researchers.³ Jors, *et al*,³⁹ found a dose-response relationship based on duration of spraying, pesticide use and use of personal protective equipment. Ciesielski, *et al*,³¹ also found this association in farm workers.

The current study examined children and adolescent pesticide applicators and a matched control group at a single time; this did not give us the chance to have a baseline measurement of AChE that is important to control for interindividual vari-

ations. Another limitation of this study was that both groups—the exposed and the control groups, were selected from the same community, which might have obscured the effect of pesticide exposure as a result of its application to cotton fields. Further research is needed to determine the effect of exposure to pesticide on developing brain and its long-term implications for health. Child labor laws are designed to reduce exposure to young adolescents. The Ministry of Agriculture should follow the child labor law which prohibits child work in those occupations with exposure to toxic substances like pesticides under the age of 17 years, and above this age workers should use the needed personal protective equipment.

Acknowledgments

We thank the participant children, adolescents and their parents for their contribution to this study. This manuscript is part of the MD thesis of Ahmed A. Ismail for his partial fulfillment of the Medical Doctorate Degree in Industrial Medicine and Occupational Health. The entire work was conducted and funded by the Faculty of Medicine, Minoufia University, Egypt.

Conflicts of Interest: None declared.

References

1. Abouleish I. The Right Livelihood Award. Sekem/Ibrahim Abouleish (Egypt). Available from <http://www.rightlivelihood.org/recipe/2003/sekem.htm>, (Accessed August 14, 2007).
2. Amr MM. Pesticide monitoring and its health problems in Egypt, a Third World country. *Toxicol Lett* 1999;**107**(1-3):1-13.
3. Kamel F, Engel LS, Gladen BC, Hoppin JA, Alavanja MC, Sandler DP. Neurologic symptoms in licensed pesticide applicators in the Agricultural Health Study. *Hum Exp Toxicol* 2007;**26**(3):243-50.
4. Smit LA, van-Wendel-de-Joode BN, Heederik D, Peiris-John RJ, van der Hoek W. Neurological symptoms among Sri Lankan farmers occupationally exposed to acetylcholinesterase-inhibiting insecticides. *Am J Ind Med* 2003;**44**(3):254-64.
5. Steenland K, Dick RB, Howell RJ, et al. Neurologic function among termiticide applicators exposed to chlorpyrifos. *Environ Health Perspect* 2000;**108**(4):293-300.
6. Misra UK, Nag D, Khan WA, Ray PK. A study of nerve conduction velocity, late responses and neuromuscular synapse functions in organophosphate workers in India. *Arch Toxicol* 1988;**61**(6):496-500.
7. Parron T, Hernandez AF, Pla A, Villanueva E. Clinical and biochemical changes in greenhouse sprayers chronically exposed to pesticides. *Hum Exp Toxicol* 1996;**15**(12):957-63.
8. Srivastava AK, Gupta BN, Bihari V, et al. Clinical, biochemical and neurobehavioural studies of workers engaged in the manufacture of quinalphos. *Food Chem Toxicol* 2000;**38**(1):65-9.
9. Stokes L, Stark A, Marshall E, Narang A. Neurotoxicity among pesticide applicators exposed to organophosphates. *Occup Environ Med* 1995;**52**(10):648-53.
10. Stallones L, Beseler C. Pesticide illness, farm practices, and neurological symptoms among farm residents in Colorado. *Environ Res* 2002;**90**(2):89-97.
11. Engel LS, Keifer MC, Checkoway H, Robinson LR, Vaughan TL. Neurophysiological function in farm workers exposed to organophosphate pesticides. *Arch Environ Health* 1998;**53**(1):7-14.
12. London L, Nell V, Thompson ML, Myers JE. Effects of long-term organophosphate exposures on neurological symptoms, vibration sense and tremor among South African farm workers. *Scand J Work Environ Health* 1998;**24**(1):18-29.
13. Abu Mourad T. Adverse impact of insecticides on the health of Palestinian farm workers in the Gaza Strip: a hematologic biomarker study. *Int J Occup Environ Health* 2005;**11**(2):144-9.
14. Ames RG, Brown SK, Mengle DC, Kahn E, Stratton JW, Jackson RJ. Cholinesterase activity depression among California agricultural pesticide applicators. *Am J Ind Med* 1989;**15**(2):143-50.
15. Ohayo-Mitoko GJ, Kromhout H, Karumba PN, Boleij JS. Identification of determinants of pesticide exposure among Kenyan agricultural workers using empirical modelling. *Ann Occup Hyg* 1999;**43**(8):519-25.
16. Chadee DD, Le Maitre A. Serum cholinesterase levels of vector control workers in Trinidad, West Indies (1979-1987). *Ann Trop Med Parasitol*

- 1991;**85**(3):345-8.
17. Farahat TM, Abdelrasoul GM, Amr MM, Shebl MM, Farahat FM, Anger WK. Neurobehavioural effects among workers occupationally exposed to organophosphorous pesticides. *Occup Environ Med* 2003;**60**(4):279-86.
 18. Patil JA, Patil AJ, Govindwar SP. Biochemical effects of various pesticides on sprayers of grape gardens. *Indian J Clin Biochem* 2003;**18**(2):16-22.
 19. Safi JM, Abu Mourad TA, Yassin MM. Hematological biomarkers in farm workers exposed to organophosphorus pesticides in the Gaza Strip. *Arch Environ Occup Health* 2005;**60**(5):235-41.
 20. Ejigu D, Mekonnen Y. Pesticide use on agricultural fields and health problems in various activities. *East Afr Med J* 2005;**82**(8):427-32.
 21. Andersen SL. Trajectories of brain development: point of vulnerability or window of opportunity? *Neurosci Biobehav Rev* 2003;**27**(1-2):3-18.
 22. Moser VC, Padilla S. Age- and gender-related differences in the time course of behavioral and biochemical effects produced by oral chlorpyrifos in rats. *Toxicol Appl Pharmacol* 1998;**149**(1):107-19.
 23. Abdel Rasoul GM, Abou Salem ME, Mechaal AA, Hendy OM, Rohlman DS, Ismail AA. Effects of occupational pesticide exposure on children applying pesticides. *Neurotoxicology* 2008;**29**(5):833-8.
 24. Weber H. [Quick and simple ultramicromethod for the determination of serum cholinesterase]. *Dtsch Med Wochenschr* 1966;**91**(43):1927-32.
 25. Tietz NW. Clinical guide to laboratory tests. 2nd ed ed. Philadelphia: W. B. Saunders 1990.
 26. Cohen J. Statistical power analysis for the behavioral sciences. 2nd ed. New York: Academic Press, 1988.
 27. Stephens R, Spurgeon A, Berry H. Organophosphates: the relationship between chronic and acute exposure effects. *Neurotoxicol Teratol* 1996;**18**(4):449-53.
 28. Rendon von Osten J, Epomex C, Tinoco-Ojanguren R, Soares AM, Guilhermino L. Effect of pesticide exposure on acetylcholinesterase activity in subsistence farmers from Campeche, Mexico. *Arch Environ Health* 2004;**59**(8):418-25.
 29. Higgins GM, Muniz JF, McCauley LA. Monitoring acetylcholinesterase levels in migrant agricultural workers and their children using a portable test kit. *J Agric Saf Health* 2001;**7**(1):35-49.
 30. Stefanidou M, Athanaselis S, Velonakis M, Pappas F, Koutselinis A. Occupational exposure to cholinesterase inhibiting pesticides: a Greek case. *Int J Environ Health Res* 2003;**13**(1):23-9.
 31. Ciesielski S, Loomis DP, Mims SR, Auer A. Pesticide exposures, cholinesterase depression, and symptoms among North Carolina migrant farmworkers. *Am J Public Health* 1994;**84**(3):446-51.
 32. Desi I, Palotas M, Vetro G, et al. Biological monitoring and health surveillance of a group of greenhouse pesticide sprayers. *Toxicol Lett* 1986;**33**(1-3):91-105.
 33. Ray PK. Pollution and Health. New Delhi: Wiley Eastern Ltd., 1992.
 34. Worthing CR. The Pesticides Manual. A World Compendium. UK: British Crop Protection Council, 1987.
 35. Issaragrisil S, Chansung K, Kaufman DW, Sirijirachai J, Thamprasit T, Young NS. Aplastic anemia in rural Thailand: its association with grain farming and agricultural pesticide exposure. Aplastic Anemia Study Group. *Am J Public Health* 1997;**87**(9):1551-4.
 36. Zayed SM, Amer SM, Nawito MF, et al. Toxicological potential of malathion residues in stored soybean seeds. *J Environ Sci Health B* 1993;**28**(6):711-29.
 37. Ilahi A, Amin N, Hashmi AS, Nawaz M, Rahman N. Incidence of endrin residues in cucumber and its effects on the biological system of rats. *J Pak Med Assoc* 1986;**36**(8):209-11.
 38. Huang NC, Lin SL, Hung YM, Hung SY, Chung HM. Severity assessment in acute paraquat poisoning by analysis of APACHE II score. *J Formos Med Assoc* 2003;**102**(11):782-7.
 39. Jors E, Morant RC, Aguilar GC, et al. Occupational pesticide intoxications among farmers in Bolivia: a cross-sectional study. *Environ Health* 2006;**5**:10.