DISCUSSION

Cholinesterase inhibitors are widely used in agriculture as insecticides, in industry, in technology as softening agents and additives to lubricants and in military technology as chemical weapons. (81)

The average annual amount of pesticides used in Egypt in official ways does not exceed 6000 tons of active ingredient. (82)

The commonly encountered OP compounds comprise insecticides (including malathion, parathion, diazinon, fenthion, dichlorvos, chlorpyrifos, ethion). Chlorpyrifos (CPF), a widely used organophosphorus pesticide (OP) in Egypt, is metabolized to CPF-oxon, a potent cholinesterase (ChE) inhibitor, and trichloro-2-pyridinol (TCPy). Whereas blood ChE activity is considered an indicator of CPF toxicity, nerve gases (including soman, sarin, tabun, VX), ophthalmic agents (echothiophate, isoflurophate), antihelmintics (such as trichlorfon), herbicides [including tribufos (DEF), merphos which are tricresyl phosphate containing industrial chemicals]. (83; 84)

Worldwide, an estimated 3,000,000 people are exposed to organophosphate or carbamates each year, with up to 300,000 fatalities. (85)

Cholinesterase enzyme inhibitors (CEIs) suppress the action of the enzyme cholinesterase and because of its essential function, chemicals that interfere with the action of cholinesterase are potent neurotoxins. Examples of CEIs are insecticides, snake venoms, and the nerve gases sarin and VX.(86)

El-Masry and Tawfik (2013),(87) reported that Poisoning Control Centre (PCC) of Ain Shams University had received 2371 cases (11% of all cases) of Organophosphorus and carbamates poisoning during the year of 2011.

Shreed et al, (2011), (88) studied the toxic agents used for parasuicide in Damietta Governorate and found that pesticides were the most common used agents (95.1%). According to Ali et al, (2007),(89) who studied the cause of unnatural deaths in elderly in Mansoura Medicolegal Centre (Ministry of Justice), carbamates poisoning (48.15%) was reported to be the most common cause of toxicological deaths followed by organophosphorus poisoning (27.77%).

They act by inhibiting the acetylcholinesterase enzyme (AchE) at muscarinic and nicotinic receptors, producing an array of symptoms like miosis, bradycardia, increased gastrointestinal motility, emesis, sweating, tachypnea, salivation, lacrimation, altered sensorium, fasciculation, bronchospasm, blurred vision, photophobia, urination and defecation. Complications include acidosis, respiratory paralysis, acute renal failure, seizures, arrythmias, aspiration, coma and even death. The causes of death in OP poisoning may be either one or a combination of the above. (83)

There are three phases of CEIs toxicity. Acute cholinergic crises (first 48-72 hours), intermediate syndrome (IMS) and delayed polyneuropathy. The acute cholinergic phase, is the most serious, usually passes off within 48-72hs but complete clinical recovery may take up to a week. Plasma cholinesterase, erythrocyte cholinesterase, and several other investigations (e.g. arterial blood gases) are mandatory in addition to physical assessment

of the case for assessment of the severity of the patients. Treatment is supportive with initial resuscitation and gastric decontamination, antidotal therapy with atropine and oximes. [90]

The aim of the current study was to investigate the validity of some indices as probable markers of severity in poisoning with cholinesterase enzyme inhibitors (CEIs) insecticides. The study was conducted on sixty patients who were admitted to the Poison Unit of Alexandria University Main Hospital, within 6 hours of exposure to CEIs insecticides without receiving any prior treatment. The study conducted from the 1st of November 2013 to the 31st of October 2014.

A clinical sheet was used to assess the patients. The following were the items used in the sheet: personal history: name, address (residency), age, gender, marital status, education, habits of medical importance (smoking), route of poisoning and circumstances of poisoning. Clinical examination: vital signs (pulse, blood pressure, respiratory rate, temperature), physical findings (pupil size), muscarinic manifestations, nicotinic manifestations, central nervous system manifestations and level of consciousness. Investigations: ECG, serum cholinesterase level, serum CPK levels (initial and final), arterial blood gases (PCO_2, HCO_3, pH). Treatment of the patients.

A) Demographic data:

In this study, the age of the patients ranged from 18 years to 52 years, with a mean of 31.7 ± 10.1 years. Mean age recorded in males was 31.9 ± 9.7 years, in comparison to females mean age 31.1 ± 11.6 years. This may reflect the more involvement of males in agricultural work at younger age than females. There was no statistically significant difference between males and females regarding the age ($\chi 2$ = 28.492, P = 0.33; t-test = 0.279, P = 0.78).

Many studies have reported similar range of age. Balouch et al, (2012),[91] recorded a mean age of 27.3 ± 8.6 years. Turabi et al, (2008),[92] reported that the age of 87.8% of the patients involved in his study ranged from 15 years to 40 years. Goel et al, (1998),[93] reported that 86.4 % of his patients ranged from 12-30 years.

In contrast to the findings of the present work, Marahatta et al, (2009),[94] reported that most of the patients were above 40 years. This difference may be due to different culture and working habits.

In the current study, males outnumbered females with male to female ratio of 2.3:1. Similar ratios were given by Thunga et al, (2010), [95] in India and Jeyaratnam et al, (1982),[96] in Sri Lanka, where the ratios were 2.1:1 and 2.6:1 respectively. Ahmed et al, at (2010),[97] Al-Minia Governorate, reported a lower ratio of 1.78:1.

In contrast to this study, some studies in Egypt reported different ratios; Mashali et al, (2005),[98] reported male to female ratio of 1:1.2. In addition, Diab, (2011),[99] found that higher numbers of patients were female (117 cases out of the 200 total cases) producing a male to female ratio of 1:1.4. Ibrahim et al, (2011),[100], conducted a study of organophosphorus-intoxicated patients admitted to intensive care unit of Ain Shams University Hospital, found that male to female ratio was 0.8:1 This may be attributed to the difference in sample size.

In Pakistan, Khan and Reza, (1998),[101] reported that about 75% of the organophosphorus-poisoned patients were under the age of 30 with predominance of females. Saadeh et al, (1996),[102] in Jordan recorded younger age predominance in cases of organophosphorus intoxication, yet with nearly equal male to female ratio (1:1.1). The difference may be attributed to different population with more predominant agricultural related accidental poisoning as 80% of patients in this study came from rural areas where increased use of OP in agriculture and the lack of protective measures resulted in this increase in accidental poisoning.

The single patients were 18 (11 males [18.3%] and 7 females [11.7%]), married patients were 20 (16 males [26.7%] and 4 females [6.7%]) and divorced patients were 22 (15 males [25%] and 7 females [11.7%]). There was no statistically significant difference between males and females regarding the marital status ($\chi^2 = 3.215$, P = 0.20).

In agreement with the current study, Iyyadurai et al, 2014 [149], found no statistically significant difference between the males and females of their study as regards the marital status.

Twenty-seven patients were illiterate, 20 patients had pre-university education and 13 patients had university education. Illiteracy may play a role in poisoning as those patients fail to read cautions on the bottles of insecticides. There was no statistically significant difference between males and females regarding the educational status ($\chi^2 = 2.148$, P = 0.34).

These data are in agreement with the previously published data of Shreed et al, (2011) [88], where they found no statistical difference as regards the educational status.

Thirty-two patients (53.3%) were farmers, 17 patients (28.3%) were students, 3 were housewives (5%) and 8 (13.4%) were laborers. There was a statistically significant difference between males and females regarding the occupation ($\chi^2 = 11.821$, P = 0.008).

These data are in agreement with the previously published data of Shreed et al, (2011) [88], where they found a statistically significant difference between males and females as regards the occupation, where most of the patients were farmers and housewives.

As regards residency, 12 patients were urban area residents (8 males 13.3% and 4 females 6.7%), however, 80% of the patients (48 patients) were rural area residents (34 males 56.7% and 14 females 23.3%). There was no statistically significant difference between males and females regarding the residency ($\chi^2 = 0.005$, P = 0.94).

It was found that 80% were from rural areas of Alexandria, Matrouh, El-Behera and Kafr El-Sheikh governorates. This may explains the high incidence of accidental intoxication among males who are usually more involved in agricultural work.

This coincides with result of Uma et al, (2011) [103], who found that 61.7% of patients with CEIs poisoning were from rural areas. In addition, Venkateswara, (2005) [104], reported that 88.8% of the patients were from rural areas. In addition, Ibrahim et al, (2011) [100], a found that 62.2% of patients were from rural areas.

In contrast to this study, Shreed et al, (2011) [88], reported that 55.9% of patients were from urban areas; this difference may be due to their selection of only para-suicidal cases.

51.6% of the patients were smokers (29 males [48.3%] and 2 females [3.3%]) while 48.4% patients were non-smokers (13 males [21.7%] and 16 females [26.7%]). There was no statistically significant difference between males and females regarding the habits (smoking) ($\chi^2 = 0.458$, P = 0.0.49).

In contrast to the previous data, Basher et al, (2013) [150], found a statistically significant difference ($p < 0.05$) as regards the smoking status between their studied subjects.

The main route of poisoning of the patients involved in the current study was the non-oral route (inhalation, transdermal), which accounted for 70% of the cases, while the oral route accounted for 30% of the cases. There was a statistically significant difference between males and females regarding the route of poisoning ($\chi^2 = 6.353$, P = 0.01).

This coincides with the fact that most of cases were accidentally exposed to pesticides, which are used during their work. Similar to the results of the present work, Shah et al, (2012) [105], reported that inhalational poisoning (54% of patients) was more common than ingestion (46% of patients). Venkateswara, (2005) [104], reported that inhalational intoxication was present in all his studied cases of accidentally intoxicated patients.

In contrast to the findings of this study, Mashali et al, (2005) [98], reported that oral route was the commonest (65%). The rest of cases (35%) were dermally intoxicated. This difference may be attributed to easy accesss to OP by females resulted in increased incidence of suicidal poisoning related to the season of this study.

World Health Organization (WHO) reports showed predominance of oral exposure to organophosphorus poisoning followed by respiratory and dermal route. [106].

This difference may be explained by the fact that this study was conducted during cropping seasons, and the majority of patients were from rural areas beside unsafe use of pesticides, which could explain the higher incidence of non-oral rote of exposure.

As regards circumstances of intoxication, 86.7% of patients were accidentally poisoned, (77% of them were males and 23% were females). There was a statistically significant difference between males and females regarding the circumstances of poisoning ($\chi^2 = 6.600$, P = 0.01).

28.6% of female patients were presented with history of suicidal attempts, while suicidal attempts among male cases represented 4.7% of them. This is consistent with higher prevalence of suicidal attempts among females than males, especially in adolescent age group. [107].

The unsafe use of pesticides by farmers may explain the high incidence of accidental intoxication and the prevalence of male gender that are more involved in manual work in the field. This was confirmed by the study done by Tchounwou et al, (2002),[108] who conducted field survey in Menia Al-Kamh province of Sharkia governorate regarding the safe use of pesticides, the study reported that 98.9 % of farmers did not wear gloves while

98.4% did not wear eye glasses or goggles when mixing or applying pesticides and 34% never wear long pants and shoes when applying pesticides.

A study conducted by Sahin et al, (2003),[109] in Turkey found a suicidal rate of 46.4% among men and 75.4% among women. Similarly, in a study to examine associations of age, gender, and psychosocial factors during adolescence with risk of suicide attempt, the suicide attempt hazard rate for female adolescents was significantly higher than for male adolescents.

Higher incidence of suicidal attempts among females more than males is agreed with Okasha et al, (1986),[110] who found that female patients are more common to commit suicide using toxic substances. This also agreed with Fahmy et al, (1996), [111] who found that female patients who attempted suicide by ingestion of toxic material represented 89.7% of cases in their study.

This result also coincides with Mosa et al, (2005) [112], who found that suicidal incidence was more pronounced in female patients (54.8%) than male patients (20.9%).

Mashali et al, (2005),[98] reported high percentage of accidental intoxication (55% of patients) and a high incidence of suicidal intoxication among females (72% of female patients) compared to suicidal intoxication among male patients (45% of male patients). [113]

B) General examination:

Vital signs:

The mean pulse rate for the whole patients was 51 ± 8 beats/minute, with a minimum of 35 beats/minute and a maximum of 66 beats/minute. Regarding the blood pressure, all patients were hypotensive (muscarinic sign) except one patient who presented with hypertension (initially hypertensive, by history). Other patients presented with average mean blood pressure (100 ± 14 mmHg).

The present study showed that the arrhythmias occurred in 81.6% of patients, where forty-four patients (73.33%) presented with sinus bradycardia that may be due to obvious muscarinic manifestations of cholinesterase inhibitors toxicity, three patients (5%) presented with sinus tachycardia and two patients (3.33%) presented with prolonged QTc interval that may be the result of intense and unequal sympathetic stimulation of myocardial fibers. The rest of the patients (11 patients; 18.33%) were presented with normal rhythm. Arrhythmias can be attributed to myocardial changes secondary to cholinergic overstimulation.

Elgendi et al, (2008),[114] found tachycardia in 6% of patients, while bradycardia was present in 62.5%, while Ahmed et al, (2010) [97], reported higher percentage of sinus tachycardia (35.93%) than bradycardia (6.23%). Ozturk et al, (1990),[115] reported that patients with OP insecticides poisoning might be presented with a spectrum of manifestations with no prevalence of muscarinic or nicotinic effects.

Pimentel and Da Costa,[116] conducted postmortem ultrastructural examination of the heart of 10 patients who died of acute poisoning with organophosphates and found lysis of myofibrils, swollen and fragmented mitochondria, disorganization of nuclear chromatin,

and Z band abnormalities. Arrhythmias may also be related to sensitization of the myocardium to circulating catecholamines from solvents such as xylene and naphtha that can be used as carriers for the organophosphorus insecticides. [39].

Respiratory manifestations of the studied patients encompassed changes in respiratory rate; tachypnea in 38.3% of cases and bradypnea in 5% of cases, while 56.7% of patients were presented with normal respiratory rate at admission. Bronchorrhea was found in all cases with variant degrees and wheezes were found in 60% of cases. These respiratory manifestations are due to combined nicotinic, muscarinic, and CNS cholinergic overstimulation which leads to broncho-constriction, bronchorrhea, and depression of the brain respiratory centre. [117]

These respiratory symptoms and signs are reported in many studies with various percentages; Diab (2011),[99] reported that 34% patients were presented with normal respiratory rates at admission, whereas, 38% of the patients were tachypneic. In addition, 28% of the cases suffered from bradypnea at admission. Bronchorrhea was found in *94.5%* of cases, wheezes were observed in 68%, pulmonary edema was observed in 31.5% of all cases. Ahmed et al (2010),[97] found bronchorrhea in 92.1% of patients.

Moreover, Elgendi et al, (2008) [114], reported that tachypnea (44%) and bronchorrhea (37.5%) were more commonly observed than bradypnea (28%). Mashali et al, (2005) [98], found that respiratory manifestations were mainly crepitations (50%), tachypnea (45%) and dyspnea (40%). While wheezes were found in 15% of cases and pulmonary edema was diagnosed in 5% of cases.

The body temperature of the patients at time of admission ranged from 35 °C to 37.4 °C with a mean of 36.1 ± 0.7 °C. None of our patients represented with evident hypothermia (< 35 °C), all patients presented with normal temperature with different degrees. Organophosphorous insecticides and other agents that cause cholinergic stimulation cause hypothermia by stimulation of inappropriate sweating and possibly through depression of the endogenous use of calorigenic substrates. [118]

This coincides with the study of Ahmed et al, (2010),[97] found hypothermia in 14.06% of patients. While Elgendi et al, (2008), [114] found hypothermia in 53.1% of patients, and the incidence of hypothermia increases with increasing severity of intoxication.

In addition, Moffatt et al, (2010) [119], found hypothermia in 50% of patients at time of admission and concluded that, in humans, OP poisoning causes an initial hypothermia, followed by a period of normal to high body temperature. These changes in body temperature may be related to atropine administration and respiratory complications.

Differences among studies regarding percentage of hypothermia may be related to the variations in severity of intoxications among patients and the difference in the ambient temperature.

Elevation in body temperature is a frequent outcome in cases of CEIs poisoning. This is most frequently encountered among cases complicated by concurrent illnesses (in particular aspiration pneumonitis/pneumonia) and interventions that may produce high temperatures, in particular anticholinergic agents. In these cases, it is unclear whether

atropine was responsible for the high temperature or whether high doses of atropine were given to patients with severe OP poisoning, and fever was a manifestation of severe OP poisoning.[120-123]

Most reports of human cases with fever following OP ingestion come from the warmer regions of the world, such as South Asia, the Middle East, and Africa. Therefore, reported fever in patients with OP poisoning may represent a loss of normal thermoregulation. [124-127]

Ocular examination:

Pupils were constricted in about 50% of the patients, pinpoint in 18.3%, while the rest were normal.

This coincides with Mokhlesi et al, (2003),[127] where miosis was present in 85% of cases with OP poisoning in his study. Miosis is a characteristic sign found in many patients with severe and moderate OP poisoning and it denotes the predominance of muscarinic effect of OP poisoning.

Muscarinic and nicotinic manifestations:

The most prevalent symptoms in the studied patients were muscarinic manifestations, vomiting (55 patients; 91.6%), followed by the most prevalent sign, bradycardia 44 patients (73.33%), salivation in 41 patients (68.3%), sweating 36 patients (60%), followed by miosis in 30 patients (50%), and the least represented symptom was tachycardia 3 patients (5%). There was no statistically significant difference between the total sample regarding the presenting symptoms ($\chi^2 = 0.036$, $P = 0.45$).

This is in agreement with Lee and Tai, (2001) [151], who revealed that cholinergic stimulation syndrome in acute OP poisoned patient, was apparent in all patients before atropinization.

POP Score:

About half of the patients (53.4%) had moderate toxicity and scored from 4 to 7, 28.3% had mild toxicity and scored from 0 – 3 all of which were males and 18.3% had severe toxicity and scored from 8 – 11 all of which were females. There was a statistically significant difference between males and females regarding the severity of poisoning ($\chi^2 = 33.958$, $P = 0.0001$).

This is in agreement with the previously reported data of Bhattacharyya et al, (2011) [128], where they used the POP Score to evaluate the severity of the patients with organophosphates poisoning, they found that 27% of their subjects were categorized as mild, 50.8% were categorized as moderate and 22.2% as severe.

Organophosphorus compounds act preferentially at the neuromuscular junctions of skeletal muscle, exerting their action both as anti-ChE agents and as direct agonists causing muscle fasciculations, cramping, weakness, and paralysis. [129]

CNS cholinergic stimulation suppresses central medullary centers accounting for depressed respirations, headache, anxiety, restlessness, confusion, psychosis, seizures, and

coma. CNS symptoms are partially due to cholinergic effects and partially to secondary changes in non-cholinergic pathways initiated within the CNS or by feedback from the periphery. [130]

Yilmazlar and Ozyurt (1997) [131], stated that the CNS effects are associated with increasing severity of the poisoning and may be linked to the cerebral perfusion defects demonstrated in these patients, especially in the parietal lobe.

Maynard and Beswick, (1992) [132], found that the muscarinic receptors responsible for CNS effects, including impairment of the respiratory center, belong to the M2 subtype, while the ones, responsible for the bronchial symptomatology (bronchospasm and bronchorrhea), are identified as Ml subtype.

Similar to the present work, Ahmed et al, (2010) [97], reported decreased level of consciousness among 51.56% of patients, convulsions in 7.8%of patients and muscle fasciculation among 34.35% of patients. Diab (2011) [99], observed twitches in 63% of all patients, convulsions in 24.5% of cases and disturbance in the level of consciousness at time of admission with mean Glasgow coma scale of 10.8 ± 2.3. Uma et al, (2011) [103], reported that convulsions had been seen in 8.3% of patients, fasciculations in 61.6% and altered level of consciousness in 26.6% of patients.

In contrast to the findings of the current study, Rehiman et al, (2008) [133], and Kumar et al, (2001) [134], did not report convulsions among their studied patients. Besides that, Kumar et al, did not report any disturbance in the level of consciousness of the studied patients.

Plasma cholinesterase level:

Regarding the investigations done to the patients, the total sample had a mean initial plasma cholinesterase level of 4140.7 ± 1099.3 U/L, which is lower than the normal range of (4900 – 11900 U/L), while they had a mean final plasma cholinesterase level of 8430 ± 2002 U/L, which is within the normal range. The comparison between the initial and the final levels of PchE was statistically significant ($p < 0.05$).

Plasma cholinesterase is one of the two kinds of cholinesterases found in humans (plasma cholinesterase [PchE] and erythrocyte-cholinesterase [EchE]); although it is considered to be less accurate than the EchE in cases of organophosphate toxicity, however, RBC cholinesterase is the more accurate of the two measurements, but plasma cholinesterase is easier to assay and is more readily available, hence, considered as a default investigation in organophosphates toxicity. [135,136].

The correlation between the on admission plasma cholinesterase levels (U/L) and POP score categorization showed that the patients with mild toxicity (0-3) had a mean PchE level of 5523.7 ± 416.6 U/L, patients with moderate toxicity (4-7) had a mean PchE level of 3968.2 ± 392.9 U/L, and patients with severe toxicity (8-11) had a mean PchE level of 2505.1 ± 304.1 U/L, with a Pearson's correlation coefficient of -0.87 (high degree of negative correlation), and a highly significant P value of < 0.0001.

Similar to the current study, Mekonnen and Ejigu, (2005),[137] conducted a study in order to study cholinesterase levels in farm workers with varying exposure to chemical

pesticide. Plasma cholinesterase (PchE) was measured in workers at two Ethiopian farms. They found that the sprayers in both farms were the most affected groups.

Similarly, a study in the Niger Delta region of Nigeria showed that the effect of organophosphorus poisoning occurs early and is more marked in plasma cholinesterase than in the red cell cholinesterase. Plasma cholinesterase is thus more useful in early detection of organophosphorus toxicity than red cell cholinesterase. [138]

However, the same authors, in another study reported that exposure to organophosphorus compounds causes reduction in the plasma cholinesterase activity and this reduction is directly related to the duration of exposure, which disagrees with the current study. [138]**.** However, cholinesterase levels do not always correlate with severity of clinical illness. Moreover, a variety of conditions can result in falsely cholinesterase levels. [136]

There are emerging options for new cheaper and/or easily quantifiable biochemical markers in relation to OP poisoning like creatine phosphokinase (CPK), lactate dehydrogenase (LDH), serum immunoglobulins (IgG, IgA), circulating complements (C3, C4), etc. However, immunoglobulin assays, apart from being costly and difficult to perform in most laboratories, are often unreliable. Several animal model studies on rat liver and fresh-water snails indicate the association between OP poisoning and CPK levels. [139;140]

The total sample had a mean initial creatine phosphokinase level of 514 ± 278.3 U/L, while they had a mean final creatine phosphokinase level of 992.9 ± 11268 U/L, both mean initial and final CPK levels are higher than the normal range (39-308 U/L males; 26-192 U/L females), the comparison between the initial and the final levels of CPK was statistically significant ($p < 0.05$).

Creatine kinase (CK), also known as creatine phosphokinase (CPK) is an enzyme expressed by various tissues and cell types. CPK catalyses the conversion of creatine and consumes adenosine triphosphate (ATP) to create phosphocreatine (PCr) and adenosine diphosphate (ADP). This CPK enzyme reaction is reversible, such that also ATP can be generated from PCr and ADP. [141]

In tissues and cells that consume ATP rapidly, especially skeletal muscle, but also brain, photoreceptor cells of the retina, hair cells of the inner ear, spermatozoa and smooth muscle, PCr serves as an energy reservoir for the rapid buffering and regeneration of ATP in situ, as well as for intracellular energy transport by the PCr shuttle or circuit. Thus, creatine kinase is an important enzyme in such tissues. [142]

The presence of muscle fiber necrosis in OP poisoning has been already demonstrated in animal experimental studies by Calore et al, (1999).[143]

Similar to the current data, a study conducted by De Wilde et al, (1991) [144], has shown that CPK was elevated in a fraction of their cases who had severe poisoning. It has been shown that there is rhabdomyolysis in "intermediate syndrome" and consequently there is raised CPK level.

De Wilde et al, (1991),[144] conducted a case report study on a 65-year-old Caucasian female who developed an intermediate syndrome seven days after an acute cholinergic crisis, caused by the ingestion of fenthion. Cholinesterase activity in the blood, plasma and

red cells was monitored daily by the method according to Nenner and serial serum fenthion levels were measured by capillary gas chromatography.

It was there hypothesized that the pathophysiologic process underlying the syndrome is the result of a time-confined phenomenon, which includes both changes in the postsynaptic structures by a desensitization process and a gradually restoring ratio of acetylcholine to acetylcholinesterase. This hypothesis was suggested by the similarity in the EMG-findings of this patient and those in myasthenia gravis, which is known to be characterized by a postsynaptic transmission defect.[128]

The correlation between the initial CPK levels (U/L) and POP score categorization among the studied patients showed that patients with mild toxicity (0-3) had a mean CPK level of 277.20 ± 91.87 U/L, patients with moderate toxicity (4-7) had a mean CPK level of 460.04 ± 80.52 U/L and patients with severe toxicity (8-11) had a mean CPK level of 1036.71 ± 134.69 U/L, with a Pearson's correlation coefficient of 0.82 (high degree of positive correlation), and a highly significant value of < 0.001.

Our study showed that serum CPK level is elevated even in the absence of intermediate syndrome, provided the patient is severely poisoned, presumably due to muscle fiber necrosis. If there is ongoing injury to the muscle due to development of complications, the CPK level continues to be elevated. Since half-life of CPK is about 1.5 days, it normalizes within 5–6 days of a single insult to the muscle. [145]

In agreement with the currently reported data, it has been shown by Senanayeke et al, (1993) [75], that the POP score can efficiently predict the severity, morbidity and mortality of OP poisoned patients. This scale uses high respiratory rate (>20/minute) and the presence of cyanosis. This approach is likely to cause difficulties as severe OP poisoning may cause either central respiratory depression with a reduced respiratory rate or tachypnea in the context of bronchorrhea, bronchoconstriction or respiratory muscle weakness. [146]

In addition, Bhattacharyya et al, (2011) [128], observed that as the POP score increases, the serum CPK value also increases and there is concomitant fall in arterial pH and increase in the total atropine requirement, which is in agreement with the current data.

Moreover, in the current study, correlation between the initial CPK levels (U/L) and PchE (U/L) and pH among the studied patients showed that inverse relationship was found between the initial CPK levels and both the plasma cholinesterase and the pH, both inverse relations were statistically significant.

The previously mentioned data are in agreement with the data reported by Bhattacharyya et al, (2011) [128], where they reported high degree of correlation between initial CPK value and PChE levels, arterial pH values and total dose of atropine (in mg). The correlation was found to be statistically significant (P < 0.001) in each case.

As regards the arterial blood gases analysis, the total sample had a mean partial carbon dioxide tension of 47.3 ± 8 mmHg, with a median of 48.7 and an interquartile range of 44-56 mmHg. The sample had a mean bicarbonate level of 23.4 ± 1.9 mEq/L, with a median of 25 and an interquartile range of 23.7-26.1 mEq/L. The mean pH level of the total sample was 7.31 ± 0.05, with a median of 7.31 and an interquartile range of 7.29-

7.35. These data show that almost ¾ of our cases suffered from uncompensated respiratory acidosis, while $^1/_3$ of the patients (36.7%) had elevated levels of HCO_3 (indicating compensated respiratory acidosis).

In contrast to the previously reported data, Basher et al, (2013) [(150)], found that the initial and final levels of arterial blood gases did not differ significantly ($p > 0.05$), which may be due to the difference in patient number (60 in our study compared to 50 patients in Basher et al, study).

The correlation between the pH levels and the POP score categorization among the studied patients, patients with mild toxicity (0-3) had a mean pH level of 7.37 ± 0.02, patients with moderate toxicity (4-7) had a mean pH level of 7.31 ± 0.02 and patients with severe toxicity (8-11) had a mean pH level of 7.23 ± 0.01, with a Pearson's correlation coefficient of – 0.86 (high degree of negative correlation), and a highly significant value of < 0.001.

The correlation between the initial PCO_2 levels (mmHg) and the POP score categorization among the studied patients. Patients with mild toxicity (0-3) had a mean PCO_2 level of 43.3 ± 7.7 mmHg, patients with moderate toxicity (4-7) had a mean PCO_2 level of 41.5 ± 6.8 mmHg and patients with severe toxicity (8-11) had a mean PCO_2 level of 42.5 ± 7.1 mmHg, with a Pearson's correlation coefficient of -0.11 (low degree of negative correlation), and a non significant P value of 0.3.

The correlation between the initial HCO_3 levels (mEq/L) and the POP score categorization among the studied patients. Patients with mild toxicity (0-3) had a mean HCO_3 level of 23.8 ± 1.8 mEq/L, patients with moderate toxicity (4-7) had a mean HCO_3 level of 23.3 ± 2 mEq/L and patients with severe toxicity (8-11) had a mean HCO_3 level of 23.3 ± 1.9 mEq/L, with a Pearson's correlation coefficient of -0.11 (low degree of negative correlation), and a non significant P value of 0.3.

The previously reported data are in agreement with the data presented by Bhattacharyya et al, (2011) [(128)], where they reported a non significant correlation between the POP score and the initial levels of both the PCO_2 and the HCO_3.

Acidosis is a frequently encountered complication of CEIs poisoning, and our study showed acidosis as a common finding. These data are in agreement with the retrospective analysis of OP-poisoned patients by Liu et al, (2008) [(147)], which found a direct correlation between the severity of poisoning and mortality and the presence of pretreatment metabolic and respiratory acidosis.

It is documented that acidosis itself can cause modest elevations in CPK levels in blood, which implies that CPK can be falsely high in case of acidosis. [(148)]**.** However, significant acidosis being a complication of severe poisoning, CPK values also correlated with degree of acidosis along with severity of poisoning.

The correlation between the initial plasma cholinesterase levels (U/L) and the initial PCO_2 levels (mmHg) among the studied patients. Patients with mild toxicity had a mean PchE level of 5523.7 ± 416.6 U/L *vs.* a mean PCO_2 of 43.3 ± 7.7 mmHg, patients with moderate toxicity had a mean PchE level of 3968.2 ± 392.9 U/L *vs.* a mean PCO_2 of 41.5 ± 6.8 mmHg and patients with severe toxicity had a mean PchE level of 2505.1 ± 304.1 U/L

vs. a mean PCO_2 of 42.5 ± 7.1, with a Pearson's correlation coefficient of –0.01 (low degree of negative correlation), and a non-significant P value of 0.9.

The correlation between the initial plasma cholinesterase levels (U/L) and the initial HCO_3 levels (mEq/L) among the studied patients, patients with mild toxicity had a mean PchE level of 5523.7 ± 416.6 U/L *vs.* a mean HCO_3 of 23.8 ± 1.8 mmol/L, patients with moderate toxicity had a mean PchE level of 3968.2 ± 392.9 U/L *vs.* a mean HCO_3 of 23.3 ± 2 mmol/L and patients with severe toxicity had a mean PchE of 2505.1 ± 304.1 U/L *vs.* a mean HCO_3 of 23.3 ± 1.9 mmol/L, with a Pearson's correlation coefficient of 0.02 (positive correlation), and a non significant P value of 0.8.

These data are in agreement with the data of Lee and Tai, (2001),[151] were they found no significant correlations between the plasma cholinesterase and the arterial blood gases in patients with acute organophosphate poisoning requiring intensive care.

Taking in consideration the fact that creatine phosphokinase is easier, cheaper and more reliable than plasma cholinesterase, hence, it is better to consider CPK as an alternative to PchE in investigating cases of CET insecticide poisoning. In addition, initial CPK levels were found to be directly and independently correlated with the levels of severity, which makes it an accurate marker for assessment of severity.

SUMMARY

CEIs insecticides poisoning (especially organophosphorus) is still a global problem especially in the developing countries as Egypt. Organophosphorus compounds are widely used as pesticides in agriculture and also as domestic insecticides. Patients may be exposed to organophosphorus compounds through inhalation, ingestion or dermal exposure.

The easy availability of these compounds and their wide spread usage makes self-poisoning by OPs compounds a common way for suicidal attacks especially in the young age group or by accidental ingestion especially in agricultural workers.

OP compounds act mainly by inhibition of acetyl cholinesterase enzyme leading to accumulation of acetylcholine and the occurrence of the characteristic signs and symptoms in the form of muscarinic, nicotinic and CNS manifestations.

Serious complications may occur from OP poisoning, one of them, is the intermediate syndrome which is characterized by muscle weakness and may result in respiratory failure and hence the need for respiratory support in form of mechanical ventilation.

Diagnosis of OP poisoning can be done by history taken from the patient or his relatives and the characteristic clinical picture.

Since the clinical picture alone is not enough to create a sufficient assessment of the patient's toxic status and severity, hence, the need for a sufficient investigations has become mandatory during the past few decades especially with the increase in the number of cases of poisoning with CEIs.

The purpose of this study was to investigate the validity of some indices as probable markers of severity in poisoning with cholinesterase enzyme inhibitors (CEIs) insecticides. The present work was carried out on 60 patients admitted to Poison Unit of Alexandria Main University Hospital from the first of November 2013 to the end of October 2014, after agreement and signing of informed consent.

A clinical sheet was used to assess the patients. The following were the items used in the sheet: personal history: name, address (residency), age, gender, marital status, education, habits of medical importance (smoking), route of poisoning and circumstances of poisoning. Clinical examination: vital signs (pulse, blood pressure, respiratory rate, temperature), physical findings (pupil size), muscarinic manifestations, nicotinic manifestations, central nervous system manifestations and level of consciousness. Investigations: ECG, serum cholinesterase level, serum CPK levels (initial and final), arterial blood gases (PCO_2, HCO_3, pH). Treatment of the patients.

Regarding the demographic data, mean age for the studied patients (n= 60) was 31.7 ± 10.1 years, with a minimum age of 18 years and a maximum age of 52 years old. 32 patients (53.3%) were farmers, 17 patients (28.3%) were students, 8 (13.3%) were laborers and 3 were housewives (5%). 12 patients were urban areas residents (8 males 13.3% and 4 females 6.7%), however, 80% of the patients (48 patients) were rural areas residents (34 males 56.7% and 14 females 23.3%). Almost all of the patients were subjected accidentally to the poisoning compounds (52 patients, 86.7%; 40 males 66.7% and 12 females 20%), however, only 8 patients were intentionally subjected to the poisoning compounds (by suicidal attempts) (8 patients, 13.3%; 2 males 3.3% and 6 females 10%).

Clinical examination of the studied patients showed that, the most prevalent symptoms in the studied patients were muscarinic manifestations: vomiting (55 patients; 91.6%), followed by the most prevalent sign, bradycardia 44 patients (73.33%), followed

by salivation in 41 patients (68.3%) then miosis in 30 patients (50%), and the least represented symptom was tachycardia 3 patients (5%). The main route of poisoning was the non-oral route (inhalation, transdermal), which accounted for 42 cases (70%; 30 males [50%] and 12 females [20%]), while the oral route accounted for 18 cases (30%; 8 males [13.3%] and 10 females [16.7%]). 17 patients (28.3%; 17 males, 0 females) were categorized as mild, 32 patients (53.4%) were categorized as moderate and 11 patients (18.3%; 0 males, 11 females) were categorized as severe.

Regarding the laboratory findings of the studied patients, the total sample had a mean initial creatine phosphokinase level of 514 ± 278 U/L, while they had a mean final creatine phosphokinase level of 992.9 ± 1126.8 U/L, both mean initial and final CPK levels are higher than the normal range (39-308 U/L males; 26-192 U/L females), the comparison between the initial and the final levels of CPK was statistically significant ($p < 0.05$).

The total sample had a mean initial plasma cholinesterase level of 4140.7 ± 1099.3 U/L, which is lower than the normal range of (4900 – 11900 U/L), while they had a mean final plasma cholinesterase level of 8430 ± 2002 U/L, which is within the normal range. The comparison between the initial and the final levels of PchE was statistically significant ($p < 0.05$).

As regards the arterial blood gases analysis, the total sample had an initial mean partial carbon dioxide tension of 47.3 ± 8 mmHg, while they had a final mean partial carbon dioxide tension of 39.9 ± 2.2. The sample had an initial mean bicarbonate level of 23.4 ± 1.9 mEq/L, while they had a final mean bicarbonate level of 24.1 ± 1.2 mEq/L. The initial mean pH level of the total sample was 7.31 ± 0.05, while the finial mean was 7.40 ± 0.02. The comparison between the initial and the final levels of all arterial blood gases was statistically significant ($p < 0.05$).

Clinical interventions with the studied patients were the total dose of atropine (mg) until the final clinical outcome was calculated for each patient. Just before discharging the patients from our hospital, the levels of serum CPK, PchE, arterial blood gases were re-evaluated and the responses were tabulated.

There was high degree of negative correlation, and a highly significant P value of < 0.0001, between the initial level of PchE (on admission) and the POP score. High degree of positive correlation, and a highly significant P value of < 0.001, between the initial level of CPK and the POP score. Inverse significant relationship was found between the initial CPK levels and both the plasma cholinesterase and the pH, both inverse relations were statistically significant.

Taking in consideration the fact that creatine phosphokinase is easier, cheaper and more effective than plasma cholinesterase, hence, it is better to consider CPK as an alternative to PchE in investigating cases of CEI insecticide poisoning. In addition, initial CPK levels were found to be directly and independently correlated with the levels of severity, which makes it an accurate marker for assessment of severity as long as exclusion criteria are considered.

CONCLUSION

Based on our study results we conclude the following:

1. There was a statistically significant difference between males and females regarding the occupation.
2. There was a statistically significant difference between males and females regarding the route of poisoning.
3. There was a statistically significant difference between males and females regarding the circumstances of poisoning.
4. There was a statistically significant difference between males and females regarding the severity of poisoning.
5. The comparison between the initial and the final levels of CPK was statistically significant.
6. The comparison between the initial and the final levels of PchE was statistically significant.
7. The comparison between the initial and the final levels of all arterial blood gases was statistically significant.
8. Correlation between the on admission plasma cholinesterase levels (U/L) and POP score categorization showed a high degree of negative correlation.
9. Correlation between the initial CPK levels (U/L) and POP score categorization among the studied patients showed a high degree of positive correlation.
10. The correlation between the initial pH levels and the POP score categorization among the studied patients showed a high degree of negative correlation.
11. The correlation between the initial CPK levels (U/L) and PchE (U/L) and pH among the studied patients showed inverse relationship between the initial CPK levels and both the plasma cholinesterase and the pH, both inverse relations were statistically significant.
12. Solitary administration of atropine in classical doses is still a recommended line of treatment of cholinesterase inhibitors toxicity. Therefore, in rural areas, administration of atropine is a sufficient line of treatment in such cases until referral to the poison centers.
13. Creatine phosphokinase (CPK) is a reliable biomarker in determining the level of organophosphates toxicity and its follow-up.