A Study on Beta-Glucuronidase Enzyme as a Probable Biomarker in Cases of Acute Poisoning by Cholinesterase Enzyme Inhibitor Insecticides

Manal H. Abd El-Aziz[1] ,Gehan Sharara[2], Asmaa S. El-Banna[1], and Shawky S. El-Naggar[3]

[1] Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine, Alexandria University
[2] Department of Biochemistry, Faculty of Medicine, Alexandria University
[3] Emergency Medicine, Al-Ameria General Hospital

Alexandria, Egypt.

Abstract Acute poisoning by cholinesterase enzyme inhibitors insecticide continues to be a detrimental problem and a potential cause of mortality especially in developing countries. Early intervention in such cases is proven to be helpful. The aim of the present work was to assess the role of serum β-glucuronidase level as probable marker for the assessment of acute cholinesterase enzyme inhibitors insecticides poisoning. ***Subjects and methods:*** the study included 60 cases of acute poisoning by cholinesterase enzyme inhibitors insecticides, admitted to the Poison unit of Alexandria Main University Hospital, another 15 healthy volunteers were included as control group. All the cases were subjected to: thorough history taking, full clinical examination, grading of poisoning severity score (PSS), laboratory investigations (serum cholinesterase activity level (CHE) and serum β-glucuronidase level (βG). ***Results:*** Serum CHE levels were significantly decreased, while Serum βG were significantly increased in the patients compared with the controls, and also, with increasing severity of poisoning. A significant negative correlation was detected between level of serum CHE and duration of hospitalization, while a significant positive correlation was found between serum level of βG and length of hospital stay. In addition, a significant inverse correlation was found between levels of CHE and βG whether in control group or in cases. Data obtained from ROC curve analysis revealed that serum βGlevel was more helpful in diagnosis of OP poisoning than CHE (accuracy 100% versus 80%), while both serum CHE andβG levels were of equal accuracy percentage for detection of poisoning severity and for prediction of outcome (100% and 91.67% each). ***Conclusion:*** SerumβG could be used as a good biomarker for the diagnosis and prognosis of cholinesterase enzyme inhibitors insecticide poisoning.

Keywords β-glucuronidase, biomarker, cholinesterase enzyme inhibitors insecticides poisoning

Introduction

Organophosphates (OPs), carbamates, and other cholinesterase inhibitors, are the most commonly used insecticides worldwide and account for more human poisonings and death annually than any other pesticide class (Watson et al., 2003).

Estimates from the WHO indicate that each year, million accidental poisonings and two million suicide attempts involving organophosphorus insecticides occur worldwide. One third of the suicides occurring globally are due to deliberate self-ingestion of pesticides (Chowdhary et al., 2014). In developing countries; exposure to insecticides constitutes one of the most common causes of poisoning. The primary reason for this is availability and easy access of pesticides together with lack of surveillance system to control its use. The mode of exposure varies including dermal, gastrointestinal and inhalational. Maximum toxic effect is through inhalation followed by gastrointestinal and dermal absorption (Kesavachandran et al., 2009).

There are more than hundred different organophosphates, which are widely used in agriculture worldwide. Organophosphates are classified as diethyl (e.g. parathion, chlorpyrifos, dichlofenthion, phorate) and dimethyl (e.g. dimethoate,

dichlorvos, malathion) pesticides (Chowdhary et al., 2014).

The toxic effect of organophosphorus insecticides are predominantly due to inhibition of acetyl cholinesterase enzyme resulting in excessive accumulation of acetylcholine at cholinergic neuro-effector junctions (Muscarinic effects), skeletal neuromuscular junction and autonomic ganglia (Nicotinic effects) as well as central effect producing cholinergic syndrome (Eddleston and Clark, 2011).

Organophosphorus poisoning can lead to severe complications as acidosis, respiratory paralysis, acute renal failure, seizures, arrhythmias, aspiration, coma and even death. Death occurs as a result of one or more of these complications. Early diagnosis of cases of organophosphorus poisoning together with accurate evaluation and appropriate treatment are often life saving (Lee and Tai, 2001). A delay of initiation of treatments limits not only outcome, but also decreases the opportunity to use oximes (cholinesterase re-activator) (Bhattacharyya et al., 2011).

Administration of the oximes is required within 24 h for effective reactivation of CHE enzyme. Therefore, as soon as acute organophosphorus poisonings diagnosed, oximes should be administered before aging of the enzyme takes place. Aging is a process in which the phosphorylated enzyme is dealkylated, and reactivation with oximes becomes impossible (Peter et al., 2006).

Egasyn-B-glucuronidase complex is located at the luminal site of liver microsomal endoplasmic reticulum. When organophosphorus insecticides (OP) are incorporated into the liver microsomes, they become tightly bound to egasyn, a carboxylesterase isozyme, and subsequently, B-glucuronidase (βG) is dissociated and released into blood (Johnson et al., 2000).

Hence, the aim of the present work was to assess the role of serum B-glucuronidase level as probable marker for the assessment of acute cholinesterase enzyme inhibitors insecticides poisoning.

Subjects and Methods

1. Subjects

The case-control study included 60 cases of acute poisoning by cholinesterase enzyme inhibitors insecticides, admitted to the Poison unit of Alexandria Main University Hospital. Another 15 age-matched healthy volunteers were included as control group. Patients selected for the study were those who were admitted within 6 hours of exposure to the poison and without receiving any prior treatment.

Exclusion criteria for selected subjects were: liver disease, severe chronic pre-existing disease, neoplasm, diabetes mellitus, or received medications.

Informed consent was obtained from all participants included in the study or from their relatives in case of comatose patients, explaining the aim and the procedure of the study. Complete confidentiality was ensured all through the study procedure. The study protocol was approved by the ethical committee of Alexandria Faculty of Medicine.

All cases were subjected to thorough history taking, full clinical examination, grading of poisoning severity score (PSS) (Eddleston et al., 2005), laboratory investigations in the form of serum cholinesterase activity level andserum B-glucuronidase level. All patients took standard treatment protocol for cholinesterase enzyme inhibitors dedicated by the patients' clinical condition.

2. Sampling and measurement of serum CHE (U/L) and serum β-glucuronidase (βG) level (ng/ml)

On admission venous blood sample was withdrawn from the patients allowing samples to clot for 2 hours at room temperature before centrifugation for 15 minutes at 1000xg. The supernatant was collected and the assays were carried out immediately. Cholinesterase activity was determined, using the colorimetric method of Ellman (Worek et al., 2012). Serum β-glucuronidase (βG) level was measured by enzyme-linked immunosorbent assay (Soltaninejad et al., 2010).

3. Statistical analysis

Data were analyzed using IBM SPSS software package version 20. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's Exact test or Monte Carlo correction. For normally distributed data, comparison between two independent populations was done using independent t-test, while more than two populations were analyzed using F-test (ANOVA) and Post Hoc test (Scheffe). Correlations between two quantitative variables were assessed using Pearson or spearman coefficients. Agreement of serum CHE and βG to differentiate between cases and control and outcome was used and was expressed in sensitivity, specificity, positive predictive value, negative predictive value and accuracy. Receiver operating characteristic curve (ROC) was plotted to analyze a recommended cutoff, the area under the ROC curve denotes the diagnostic performance of the test. Area more than 50% gives acceptable performance and area about 100% is the best performance for the test. Significance of the obtained results was judged at the 5% level (Kirkpatrick and Feeney, 2013).

Results

Age and sex

The present study showed that 53.3% of cases were females (n=32) and 46.7% were males (n=28), with female to male sex ratio of 1.14: 1. The youngest age encountered was one year and the oldest age was 40 years with a mean age of 17.4 ± 10.03 years. There was no statistical significant difference between males and females regarding their age (P> 0.05) (Table 1).

Circumstances of poisoning

The present study showed that the rate of suicidal poisoning was higher than accidental one. A statistically significant difference was found between both sexes regarding the circumstances of poisoning, where X^2=6.461, P <0.011.The majority of females (78.1%) took the poison intentionally (suicidal), while there was a higher incidence of accidental exposure (53.6%) than suicidal one (46.4%) in males (Table 2).

In addition, the present study showed a statistically significant association between age and circumstances of poisoning, where χ^2=39.486, P=<0.001. None of suicidal cases occurred at age group below 10 years, while half of suicidal cases were in age group from 20 years to less than 30 years, followed by cases in age group from 10 to less than 20 years which constituted 39.5% (Table 3).

Clinical assessment

The studied patients were divided according to PSS into three grades, 50% of patients (n=30) had score 1 (mild poisoning), 20 patients (33.3%) had score 2 (moderate poisoning), 10 patients (16.7%) with score 3 (severe poisoning) (Figure 1).

Laboratory investigations

The current work revealed that in control group, the levels of serum CHE ranged from 5300 – 12900 U/L with a mean value of 9345.33 ± 2406.06U/L and the levels of βG ranged from 30 – 1.20 ng/ml, with a mean value of 0.71 ± 0.27 ng/ml. While in cases, the levels of serum CHE ranged from 600-11370 U/L with a mean value of 4306.87 ± 3234.82U/L and the levels of βG ranged from 3.22 – 10 ng/ml, with a mean value of 6.17 ± 2.03 ng/ml. Serum CHE levels were significantly decreased, while Serum βG were significantly increased in the patients compared with the controls (where, z=4.537, P<0.001 and t= 20.124, P<0.001 respectively) (Table 4).

ROC curve for the diagnostic performance of serum CHE and βG

The present study showed that CHE value that was equal or less than the cutoff value of 6996U/L was sensitive and specific in the diagnosis of OP poisoning, at sensitivity of78.33%, specificity of 86.67% and accuracy of 80%. While βG value over the cutoff value of 1.2 ng/ml was sensitive and specific in the diagnosis of OP poisoning, at sensitivity of100%, specificity of 100%and accuracy of 100% (Table 5).

Relation between PSS and levels of serum CHE and serum βG

Table (6) illustrates that there was a statistically significant difference among three grades of poisoning severity regarding serum level of CHE cases, where F = 88.42 and P < 0.001. Cases with PSS 1 (mild poisoning) had the highest levels of serum CHE, which was significantly higher than the other two grades of severity (PSS 2 and 3) using Post Hoc test (Scheffe), where P < 0.001. In addition, there was a highly significant negative correlation between level of CHE and severity of poisoning, where, r = -0.912 and P < 0.001. On the other hand, the lowest βG values was reported in patients with mild poisoning (PSS1) which were significantly increased with increase the grade of poisoning severity, where F=207.937 and P < 0.001.There was a highly significant positive correlation between level of βG and severity of poisoning, where, r = 0.913 and P < 0.001.

On comparing the enzymatic status in mildly poisoned patients (PSS1 to that of healthy controls, the present study revealed that the levels of CHE were significantly decreased, while βG values were significantly increased (p < 0.01) in mildly poisoned patients. CHE levels in healthy control were 1.32times higher than mildly poisoned patients. At the same time, βG levels were 6.22 times higher in mildly poisoned patients as compared to those in healthy controls (Table 7).

In addition, the activity of β–glucuronidase were significantly elevated (p < 0.01) in moderately poisoned patients (PSS 2) compared to the controls, where it increased 10.18 times and 1.63times as compared to healthy controls and PSS1 patients respectively (Table 7).

The same, the activity of β–glucuronidase were significantly increased (p < 0.01) in severely poisoned patients (PSS3). In severely poisoned patients the activity of β–glucuronidase was 13.15 times higher as compared to controls and 1.29times higher as compared to PSS 2 patients (Table 7).

ROC curve for serum CHE and βG for detection of poison severity

Table (8) illustrates that CHE value that was equal or less than the cutoff value of 1050U/L was sensitive and specific in the diagnosis of severe OP poisoning. Moreover, CHE value over the cutoff value of 3996 U/L was sensitive and specific in the diagnosis of mild OP poisoning, while CHE value lying in between these two cutoff values was sensitive and specific in the diagnosis of moderate cases of poisoning. All these results were at sensitivity, specificity and accuracy of 100%.

ROC curve also revealed that βGvalue over cutoff value of 8.65ng/ml was sensitive and specific in the diagnosis of severe OP poisoning. Moreover, βG value that was equal or less than the cutoff value of 5.7 ng/ml was sensitive and specific in the diagnosis of mild OP poisoning, while βG value lying in between these two cutoff values was sensitive and specific in the diagnosis of moderate cases of poisoning. Also, all these results were at sensitivity, specificity and accuracy of 100%.

Duration of hospitalization and outcome

Regarding the length of stay of patients in hospital in the current work, it ranged from one to 18 days, with a mean of 4.38 ± 4.03 days. Complete recovery without sequelae occurred in 95% of cases (n= 57), while death occurred in 5% of cases (n=3), 2 on day 15 and the third one on day 18 after poisoning. The cause of death appeared to be cardio-respiratory failure.

Table (9) shows that there was a statistically significant association between length of hospitalization and grades of poison severity,

where$^{KW}\chi^2$ = 44.868 and P <0.001.The duration of hospitalization was significantly increased in moderate cases than mild ones and in severe cases than moderate and mild ones using Mann Whitney test.

Table (10) illustrates the significant negative correlation between level of serum CHE and duration of hospitalization, where r = -0.799 and P <0.001. On the other hand, it shows the significant positive correlation between serum level ofβG and length of hospital stay.

Table (11) illustrates that there was a statistically significant association between outcome and grades of poison severity, where$^{FE}\chi^2$ = 15.789 and P = 0.003. The three dead cases were having the PSS 3 (severe grade), there was a statistically significant difference between PSS 3 and both PSS1 and PSS 2 using Chi square test, where p ≤ 0.05.

ROC curve for serum CHE and βG for prediction of outcome

The present study showed that CHE value that was equal or less than the cutoff value of 849U/L was sensitive and specific in the prediction of outcome of OP poisoning, at sensitivity of100%, specificity of 91.23% and accuracy of 91.67%. While βG value over the cutoff value of 8.9 ng/ml was sensitive and specific in the prediction of outcome of OP poisoning, at sensitivity of100%, specificity of 91.23% and accuracy of 91.67% (Table 12).

Correlation between levels of CHE and βG in each studied group

Table 13 shows that there was a significant inverse correlation between levels of CHE and βG whether in control group or in cases, where r=-0.639, P= 0.01 and r =-0.915, p<0.001 respectively).

Table (1): Percentage and relation between sex and age in studied cases (n=60)

	Male (n=28)		Female (n=32)		Total (n=60)	
	No.	%	No.	%	No.	%
Age						
0 - <10	8	28.6%	7	21.9%	15	25%
10 - <20	5	17.9%	11	34.4%	16	26.7%
20 - <30	10	35.7%	12	37.5%	22	36.7%
30 – 40	5	17.9%	2	6.3%	7	11.7%
χ^2	3.445					
^{MC}P-value	0.330 (NS)					
Min. – Max.	1.0 - 40.0		2.0 - 31.0		1.0 – 40.0	
Mean ± SD.	18.61±11.56		16.35 ± 8.52		17.4 ± 10.03	
Median	20.0		18.0		19.0	
Z	0.823					
P-value	0.410 (NS)					

χ^2: for Chi square test, MC: Monte Carlo test; Z: Z for Mann Whitney test; NS: Non significant

Table (2): Statistical analysis (X^2) between sex and circumstances of poisoning in studied cases (n=60)

	Male (n=28)		Female (n=32)		Total (n=60)	
	No.	%	No.	%	No.	%
Circumstances of poisoning						
Accidental	15	53.6%	7	21.9%	22	36.7%
Suicidal	13	46.4%	25	78.1%	38	63.3%
χ^2	6.461*					
^{MC}p	0.011*					

χ2: value for Chi square; MC: Monte Carlo test; *: Statistically significant at p ≤ 0.05

Table (3): Statistical analysis (X^2) between age and circumstances of exposure in cases group

	Circumstances of exposure				Total (n=60)	
	Accidental (n=22)		Suicidal (n=38)			
	No.	%	No.	%	No.	%
Age						
0 - <10	15	68.2%	0	0.0%	15	25%
10 - <20	1	4.5%	15	39.5%	16	26.7%
20 - <30	3	13.6%	19	50%	22	36.7%
30 – 40	3	13.6%	4	10.5%	7	11.7%
χ^2	39.486*					
^{MC}p	<0.001*					

*χ2: value for Chi square, MC: Monte Carlo test; *: Statistically significant at p ≤ 0.05*

Table (4): Statistical analysis (t-test) for comparison between cases and control according to serum CHE, serum βG

	Cases (n=60)	Control (n=15)	Test of sig.	p
Serum CHE(U/L)				
Min. – Max.	600– 11370	5300– 12900	t=4.537*	<0.001*
Mean ± SD.	4306.87 ± 3234.82	9345.33 ± 2406.06		
Median	3996.0	9200.0		
Serum βG(ng/ml)				
Min. – Max.	3.22 – 10	0.30 – 1.20	t=20.124*	<0.001*
Mean ± SD.	6.17 ± 2.03	0.71 ± 0.27		
Median	5.75	0.70		

*t: Student t-test; *: Statistically significant at p ≤ 0.05*

Table (5): Agreement (sensitivity, specificity and accuracy) for serum CHE and βGwith both studied groups (for the diagnostic performance of serum CHE and βG with studied groups)

		Sensitivity	Specificity	PPV	NPV	Accuracy
Serum CHE (U/L)	>6996	78.33	86.67	95.92	50.0	80.0
	≤6996					
Serum βG (ng/ml)	≤1.2	100.0	100.0	100.0	100.0	100.0
	>1.2					

Table (6): Correlation between PSS and levels of serum CHE and serum βG

	PSS		
	1 Mild (n=30)	2 Moderate (n=20)	3 Severe (n=10)
Serum CHE (U/L)			
Min. - Max.	3996.0–11370.0	1086.0–3996.0	600.0–1050.0
Mean ± SD.	7072.83±2167.18	1902.75±861.77	817.20±139.41
Median	6996.0	1524.0	801.0
F (p)	88.421* (<0.001*)		
Sig. bet. PSS grades	I-II***,I-III***		
r_s (p)	-0.912* (<0.001*)		
Serum βG (ng/ml)			
Min. - Max.	3.22 – 5.70	5.80 – 8.65	8.75 – 10.0
Mean ± SD.	4.42 ± 0.58	7.23 ± 0.98	9.34 ± 0.40
Median	4.25	7.20	9.30
F (p)	207.937*(<0.001*)		
Sig. bet. PSS grades	I-II***,I-III***,II-III***		
r_s (p)	0.913* (<0.001)		

*F: F test (ANOVA); r_s: Spearman coefficient; Sig. bet. PSS grades were done using Post Hoc test (Scheffe); *: Statistically significant at p ≤ 0.05; ***: Statistically significant at p ≤ 0.001*

Table (7): Levels of serum CHE and serum βG in controls and 3 grades of poisoning

Parameters	Control (n=15)	PSS		
		1 Mild (n=30)	2 Moderate (n=20)	3 Severe (n=10)
CHE	9345.33 ± 2406.06	7072.83±2167.18	1902.75±861.77	817.20±139.41
F	78.862*			
P-value compared to control	-	<0.001*	<0.001*	<0.001*
r_s (p)	-0.879*(<0.001)			
B- gluc	0.71 ± 0.27	4.42 ± 0.58	7.23 ± 0.98	9.34 ± 0.40
F	445.664*			
P-value compared to control	-	<0.001*	<0.001*	<0.001*
r_s (p)	0.952*(<0.001)			

*F: F test (ANOVA); r_s: Spearman coefficient; *: Statistically significant at $p \leq 0.001$*

Table (8): Agreement (sensitivity, specificity and accuracy) for serum CHE and βG with PSS

Cutoff value	Mild	Moderate	Severe	Sensitivity	Specificity	PPV	NPV	Accuracy
Serum CHE (U/L)	>3996	>1050 - 3996	≤1050	100.0	100.0	100.0	100.0	100.0
SerumβG (ng/ml)	≤5.7	>5.7 – 8.65	>8.65	100.0	100.0	100.0	100.0	100.0

Table (9): Relation between PSS and duration of hospitalization

	PSS			Total (n=60)
	1 Mild (n=30)	2 Moderate (n=20)	3 Severe (n=10)	
Duration of hospitalization				
Min. - Max.	1.0 – 7.0	3.0 – 8.0	3.0 – 18.0	1.0 – 18.0
Mean ± SD.	1.63 ± 1.13	5.25 ± 1.74	10.90 ± 4.70	4.38 ± 4.03
Median	1.0	5.0	11.50	3.0
$^{KW}\chi^2$ (p)	44.868* (<0.001*)			
Sig. bet. PSS grades	I-II***,I-III***,II-III**			

*$^{KW}\chi^2$: Chi square test value for Kruskal Wallis test; Sig. bet. PSS grades was done using Mann Whitney test; *: Statistically significant at $p \leq 0.05$; **: Statistically significant at $p \leq 0.01$; ***: Statistically significant at $p \leq 0.001$*

Table (10): Correlation between length of hospital stay with BG and CHE in cases group

	Length of hospital stay	
	r_s	p
Serum CHE	-0.779*	<0.001
Serum BG	0.788*	<0.001

*r_s: Spearman coefficient; *: Statistically significant at $p \leq 0.05$*

Table (11): Relation between PSS and outcome

	PSS						Total (n=60)	
	1 Mild (n=30)		2 Moderate (n=20)		3 Severe (n=10)			
	No.	%	No.	%	No.	%	No.	%
Outcome								
Recovery	30	100.0	20	100.0	7	70.0	57	95.0
Death	0	0.0	0	0.0	3	30.0	3	5.0
χ^2 (^{FE}p)	15.789* (0.003*)							
Sig. bet. PSS grades	I-III*,II-III*							

*χ^2: value for Chi square; FE: Fisher Exact test; Sig. bet. PSS grades were done using Chi square; *: Statistically significant at $p \leq 0.05$; ***: Statistically significant at $p \leq 0.001$*

Table (12): Agreement (sensitivity, specificity and accuracy) for serum CHE and βGwith patients' outcome.

		Recovery	Death	Sensitivity	Specificity	PPV	NPV	Accuracy
Serum CHE (U/L)	>849	52	0	100.0	91.23	37.50	100.0	91.67
	≤849	5	3					
Serum βG (ng/ml)	≤8.9	52	0	100.0	91.23	37.50	100.0	91.67
	>8.9	5	3					

Table (13): Correlation between levels of CHE and βG in each studied group

	Levels of CHE versus βG	
	r	p
Cases group	-0.915[*]	<0.001
Control group	-0.639[*]	0.010

*r: Pearson coefficient; *: Statistically significant at p ≤ 0.05*

Table (14): Accuracy, sensitivity and specificity for diagnostic and prognostic performance of both studied biomarkers (n=60).

Parameters	Cutoff point	Overall accuracy %	Sensitivity	Specificity
Serum CHE (U/L)	Cases ≤6996<Control	80%	78.33%	86.67%
Serum βG (ng/ml)	Control ≤1.2<Cases	100%	100%	100%
Serum CHE (U/L)	Mild>3996 Moderate>1050-3996 Severe ≤1050	100%	100%	100%
Serum βG (ng/ml)	Mild ≤5.7 Moderate>5.7-8.65 Severe>8.65	100%	100%	100%
Serum CHE (U/L)	Death ≤849<Recovery	91.67%	100%	91.23%
Serum βG (ng/ml)	Recovery ≤8.9<Death	91.67%	100%	91.23%
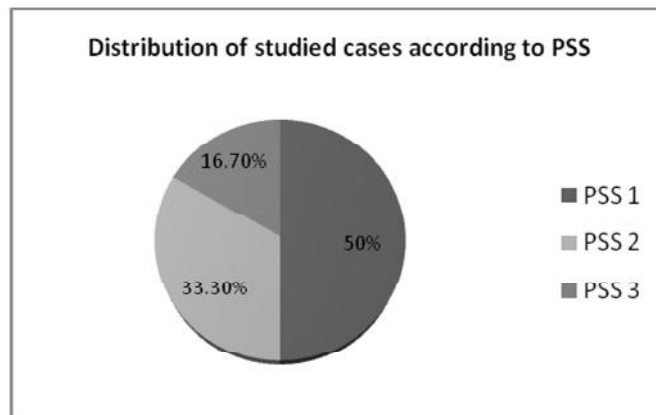
Figure (1): Distribution of studied cases according to poison severity score.

Discussion

Organophosphate (OP) poisoning is an important clinical problem in the developing world especially in rural regions, and severe acute OP poisoning is a medical emergency (Eddleston et al., 2008). The mortality rate for OP poisoning is high, with fatality often related to a delay in diagnosis or improper management (Sungur and Guven, 2001).

The present study showed that 53.3% of cases were females (n=32) and 46.7% were males (n=28). The same finding was reported by Akdur et al., (2010). The mean age of patients was 17.4 ± 10.03 years which is consistent with Khan and Reza, (1998) who reported

that most OP patients were under the age of 30 years with predominance of females.

The present study showed that the rate of suicidal poisoning was higher than accidental one, this may be explained by cheap price and uncontrolled sale of insecticide over the counter in developing countries. A statistically significant difference between both sexes regarding the circumstances of poisoning was found, where the majority of females (78.1%) took the poison intentionally (suicidal). women tend to use less lethal means of suicide which carry a higher chance of survival and they more commonly express an appeal

for help by conducting the attempt in a manner that favors discovery and rescue.(Hanna et al, 2011)Half of suicidal cases were in age group from 20 years to less than 30 years and 39.5% of suicidal cases were in age group from 10 to less than 20 years. This may be attributed to the emotional instability and problems facing teenagers and youth.

In modern clinical medicine, the search for specific sensitive biomarkers for syndromes is increasing. These biomarkers are mainly focusing to confirm diagnosis, predict outcome or to add value in the follow-up. In clinical practice, diagnosis of an acute organophosphorus (OP) poisoning depends mainly on history of exposure, physical manifestations, clinical suspicion, and laboratory support. Until now, measuring the acetyl cholinesterase activity in the red blood cells was considered to be the standard to evaluate the biochemical effects and severity following an acute and chronic OP overdose (Lockridge and Schopfer, 2006). However, this technique needs expertise, and in many clinical laboratories, pseudocholinesterase activity is measured because of its simplicity and relative accuracy.

Plasma CHE is subject to variations induced by hereditary deficiency of the enzyme, liver dysfunction, malnutrition, and drugs such as cocaine and opioids and thus less specific. Additionally, day-to-day variation in the activity of this enzyme in healthy individuals may be as high as 20% (Eddleston and Clark, 2011). Furthermore, intra-individual variation of blood cholinesterase activity has been reported as 6.3% for men and 6.7% for women respectively (Chowdhary et al., 2014). Therefore, a search for a more sensitive and specific biomarker for such a potentially severe poisoning is of health care benefit especially that OP patients admitted in good condition can be rapidly deteriorated requiring intubation and mechanical ventilation (Sungurtekin et al., 2006).

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. But the main disadvantage with these markers is their non-specificity. Moreover, immunoglobulin assays, apart from being costly and difficult to perform in most laboratories, are often unreliable (Bhattacharyya et al., 2011). Yarden et al., (2013) suggested that S100B protein may be a useful marker in assessment of clinical severity and predicting mortality of OP poisoning.

In the present study, there was a statistically significant difference between cases and control regarding both CHE and βG levels in serum.

Cholinesterase activity was decreased to approximately 50% of the control level, while plasma BG was significantly increased to be approximately 8.5 times the control level. This indicates that increase of plasma BG activity is much more susceptible than cholinesterase inhibition to insecticides.

Satoh et al., (1999) also stated that increase of the plasma BG level after OP administration is much more sensitive biomarker than cholinesterase inhibition to acute intoxication of OPs and carbamates as the change occurred in BG level after exposure was much higher than that occurred in CHE level (150% versus 50%).

CHE value that was equal or less than the cutoff value of 6996U/L was sensitive and specific in the diagnosis of OP poisoning with accuracy of 80%. While βG value over the cutoff value of 1.2 ng/ml was sensitive and specific in the diagnosis of OP poisoning with accuracy of 100%. Sensitivity means (true positive) which is more important and has higher value than the specificity which is (true negative). These data suggest thatβGwas more helpful in diagnosis of OP poisoning than CHE.

Fujikawa et al., (2005) reported that β-glucuronidase was a much more sensitive biomarkerfor acute OP exposure than acetyl cholinesterase inhibition. Where, a single administration of EPN (O-ethyl O-p-nitrophenyl phenyl phosphonothioate) and chlorpyrifos increased plasma βG activity in approximately 100-fold the control level (peak time of increase BG levels was between 2-5 hours after OP treatment). However, these reported findings were produced using a rat model.

Serum CHE values were significantly decreased with increase the grade of poisoning severity. A highly significant negative correlation was detected between level of CHE and severity of poisoning in the present study. This is in agreement with Kandalaft et al., (1991).

In the current study, βG values were significantly increased with increase the grade of poisoning severity. A highly significant positive correlation was detected between level of βG and severity of poisoning. Similarly, Kale, (2013) found a proportional increase in (βG) activity with severity of organophosphorus poisoning which will be helpful in the diagnosis and prognosis of organophosphorus poisoning.

On contrary, Sabbe et al., (2008) reported that on taking serial samples for measurement of both serum CHE and βG, the serum CHE activity supported the clinical findings and helped to decide when the patient could be weaned of the ventilator. While, β-glucuronidase activity surprisingly decreased with time, but within the normal range.

Similarly, BG activity in experimental animals was found to increase rapidly to a maximum level about 1 h after exposure and returned to a baseline level within 24 h (Kikuchi et al., 1981). In contrast, for an OP-induced decrease in serum CHE activity, it occurred immediately after high dose OP

exposure and took more than 20–30 days to recover to the base level (O'Malley, 2007). These findings raised the possibility that an OP-induced change in BG activity might reflect more recent OP exposure compared with blood CHE activity. This advantage of BG makes it more helpful in determining the efficacy of oxime administration.

On comparing the enzymatic status in mildly poisoned patients (PSS1 to that of healthy controls, the present study revealed that the levels of CHE were significantly decreased, while βG values were significantly increased (p < 0.01) in mildly poisoned patients. CHE levels in healthy control were 1.32times higher than mildly poisoned patients. On the other hand, βG levels were 6.22times higher in mildly poisoned patients as compared to those in healthy controls. So, the present work revealed that (βG) activity was helpful in diagnosis of low dose (mild) poisoning.

Ueyama et al., (2010) concluded that plasma βG activity is more sensitive biomarker as well as urinary OP metabolites than serum CHE for low-level exposure in humans. On contrary, Soltaninejad et al., (2007) observed a significant increase in blood BG activity only in patients severely intoxicated by OP exposure compared to that in controls and this may be explained by their small sample size (22 patients).

The data obtained from ROC curve analysis suggest that both serum CHE and βGwere equal in predicting the severity of OP poisoning (table 8). Yarden et al., (2013) stated that serum CHE level is related to the clinical severity but not with mortality. In contrast, other previous studied reported that low serum CHE support the diagnosis of OP poisoning but are not significantly related to the severity of poisoning (Aygun et al., 2002, Yun et al., 2012). These different results may be associated with the different grading systems used in those studies.

There was a significant increase in length of hospital stay of patients with increased poisoning severity in the current study. This reflects the socioeconomic aspect associated with organophosphorus intoxication especially in severe cases resulting in increased cost of treatment and days of absence from work.

The present study showed a significant negative correlation between level of serum CHE and duration of hospitalization. On the other hand, it showed a significant positive correlation between serum level of βG and length of hospital stay.

A significant mortality prediction was detected with increased severity of poisoning in the studied patients. All mortality cases had the highest grade of severity (PSS 3). This is consistent with results reported by Tsao et al., (1990) and Cunha et al., (1995).

The present study showed that CHE value that was equal or less than the cutoff value of 849U/L was sensitive and specific in the prediction of outcome of OP poisoning with accuracy of 91.67%. While βG value over the cutoff value of 8.9 ng/ml was sensitive and specific in the prediction of outcome of OP poisoning with accuracy of 91.67%. These data suggest that both CHE and βGwere equal in predicting the outcome of OP poisoning.

In addition, the current work revealed that there was an inverse correlation between levels of CHE and βG whether in control group or in cases.

Consequently, the increase in plasma βG activity could be a good biomarker of cholinesterase enzyme inhibitor insecticides exposure even in mild cases of poisoning.

Recommendation

The present work recommends for making researches which include dose-response relationship between OP exposure and BG activity in the human body, due in part to the difficulty in determining the exact OP exposure level and elapsed time after the exposure, plotting time–activity curve of BG activity after OP exposure in the human body.

References

Akdur O, Durukan P, Ozkan S, et al. (2010): Poisoning severity score, Glasgow coma scale, corrected QT interval in acute organophosphate poisoning. Hum Exp Toxicol. 32 (10): 1081-8.

Aygun D, Doganay Z, Altintop L, et al. (2002): Serum acetylcholinesterase and prognosis of acute organophosphate poisoning. J ToxicolClin Toxicol.40: 903–10.

Bhattacharyya K, Phaujdar S, Sarkar R, et al. (2011): Serum creatine phosphokinase: A probable marker of severity in organophosphorus poisoning.Toxicol Int. 18(2): 117–23.

Chowdhary S, Bhattacharyya R and Banerjee D (2014): Acute organophosphorus poisoning. ClinicaChimicaActa. 431: 66–76.

Cunha J, Póvoa P, Mourão L, et al. (1995): Severe poisoning by organophosphate compounds. An analysis of mortality and of the value of serum cholinesterase in monitoring the clinical course. Acta Med Port.8(9):469-75.

Eddleston M and Clark RF (2011): Insecticides: organic phosphorus compounds and carbamates. In: Goldfrank's toxicologic emergencies, Nelson LS, Lewin NA and Howland MA (eds), 9th ed ,McGraw-HillNew York, NY, pp. 1450–6.

Eddleston M, Buckley NA, Eyer P, et al., (2008): Management of acute organophosphorus pesticidepoisoning. Lancet 371: 597–607.

Eddleston M, Eyer P, WorekF, et al., (2005): Differences between organopphosphorus

insecticides in human self poisoning: a prospective cohort study. Lancet 366: 1452- 9.

Fujikawa Y, Satoh T, Suganuma A, et al.(2005): Extremely sensitive biomarker of acute organophosphorus insecticide exposure. Human and Experimental Toxicology. 24(6): 333-6.

Hanna MM, El-Shereef E, Azza H. et al. (2011): Study of pattern and outcome of suicidal methods usedin Benghazi City – Libya. Egyptian Journal of Forensic Sciences. 1:124–32.

Johnson MK, Jacobsen D and Meredith TJ (2000): Evaluation of antidotes for poisoning by organophosphorus pesticides. Emerg Med. 12: 22-37.

Kale B(2013): β- Glucuronidase as a new potent biomarker in the diagnosis and prognosis of organophosphorus toxicity. International Journal of Biological Research. 1 (1): 5-9.

Kandalaft K, S Liu S, Manivel C (1991): Organophosphate increases the sensitivity of human exocrine pancreas to acetylcholine. Pancreas. 6:398-403.

Kesavachandran CN, Fareed M, Pathak MK, et al. (2009): Adverse health effects of pesticides in agrarian populations of developing countries. Rev Environ ContamToxicol.200:33–52.

Khan MM and Reza H (1998): Gender differences in nonfatal suicidal behavior in Pakistan: significance of sociocultural factors. Suicide Life Threat Behav. 28(1):62-8.

Kikuchi H, Suzuki Y and Hashimoto Y (1981): Increase of beta-glucuronidase activity in the serum of rats administered organophosphate and carbamate insecticides. J. Toxicol. Sci.6: 27–35.

Kirkpatrick LA and Feeney BC (2013): A simple guide to IBM SPSS statistics for version 20. Student ed., Wadsworth, Cengage Learning, Belmont, Calif.

Lee P and Tai Dy (2001): Clinical features of patients with acute organophosphorus poisoning requiring intensive care. Intensive Care Med. 27(4):694-9.

Lockridge O andSchopfer LM (2006): Biomarkers of organophosphate exposure. In: Toxicology of Organophosphate and Carbamate Compounds Gupta, RC (ed), Academic Press/Elsevier , Amsterdam, p. 703–11.

O'Malley MA (2007): Pesticide. In: Current Occupational and Environmental Medicine LaDou, J. (Ed.), 4th ed. McGraw-Hill, New York, p. 544.

Peter JV, Moran JL, Graham P (2006): Oxime therapy and outcomes in human organophosphate poisoning: an evaluation using meta-analytic techniques. Crit Care Med. 34:502–10.

Sabbe MB1, Desruelles D, Lissens W (2008): Is beta-glucuronidase a clinical useful biomarker for an acute organophosphorus poisoning? Hum Exp Toxicol. 27(5): 431-3.

Satoh T, Suzuki S, Kawai N, et al.(1999): Toxicological significance in the cleavage of esterase-beta-glucuronidase complex in liver microsomes by organophosphorus compounds. Chem-Biol Interact.119–120: 471–8.

Soltaninejad K, Shadnia S, Afkhami-Taghipour M, et al. (2010): Laboratory of Forensic Toxicology, Legal Medicine Organization, Tehran, Iran. Toxicol Lett. 193(1):115-9.

Soltaninejad K, Shadnia S, Afkhami-Taghipour M, et al.(2007): Blood beta-glucuronidase as a suitable biomarker at acute exposure of severe organophosphorus poisoning in human. Human and Experimental Toxicology 26: 963–6.

Sungur M and Guven M (2001): Intensive care management of organophosphate insecticide poisoning. Crit Care 5: 211–5.

Sungurtekin H, Gurses E and Balci C (2006): Evaluation of several clinical scoring tools in organophosphate poisoned patients. Clin Toxicol (Phila). 44:121–6.

Tsoa ICY, Juang YC, Lan RS, et al. (1990): Respiratory failure of acute organophosphate and carbamate poisoning. Chest 98:631-36.

Ueyamaa J, Satoh T, Kondo T, et al. (2010): β-Glucuronidase activity is a sensitive biomarker to assess low-level organophosphorus insecticide exposure. Toxicology Letters 193: 115–9.

Watson WA, Litovitz TL and Rodgers CG (2003):2002 annual report of the American Association of Poison Control Centers Toxic Exposure Surveillance System. Am J Emerg Med. 21(5): 353-421.

Worek F, Eyer P, and Thiermann H (2012): Determination of acetyl cholinesterase activity by the Ellman assay: a versatile tool for in vitro research on medical counter measures against organophosphate poisoning. Drug Test Anal.4:282–91.

Yardan T, Baydin A, Acar E, et al.(2013): The role of serum cholinesterase activity and S100B protein in the evaluation of organophosphate poisoning. Human and Experimental Toxicology. 32(10) 1081–8.

Yun HW, Lee DH, Lee JH, et al.(2012): Serial serum cholinesterase activities as a prognostic factor in organophosphate poisoned patients. Hong Kong J Emerg Med. 19: 92–7.

<u>الملخص العربي</u>

دراسةعلي إنزيم بيتا جلكيورينيداز كعلامة حيوية محتملة في حالات التسمم الحاد بالمبيدات المثبطةلإنزيم كولينيستيريز

منال حسن عبد العزيز¹ و جيهان شرارة² و أسماء سعيد البنا¹ و شوقي سعد النجار³

يعتبر التسمم بالمبيدات المثبطة لإنزيم كولينيستيريز مشكلةخطيرة و سبب محتمل للوفاة خاصة في الدول النامية. التدخل المبكر في العلاج في مثل هذه الحالات قد أثبت أن يكون معاونا على الشفاء. كان الهدف من هذه الدراسة هو تقييم دور قياس مستوى إنزيم بيتاجلكيورينيداز كعلامة حيوية محتملة في تقييم التسمم الحاد بالمبيدات المثبطة لإنزيم كولينيستيريز. اشتملت الدراسة على ٦٠ حالة من التسمم بالمبيدات المثبطة لإنزيم كولينيستيريز والذين أدخلوا في وحدة السموم بالمستشفى الرئيسي الجامعي بالإسكندرية، كما اشتملت على ١٥ من المتطوعين الأصحاء كمجموعة ضابطة. تم أخذ تاريخ مرضى شاملمن جميع الحالات و أجري عليها كشف إكلينيكي شامل، كما تم حساب درجة شدة التسمم لهم وعمل الفحوصات المعملية متمثلة في إنزيم كولينيستيريز و إنزيم بيتا جلكيورينيداز بمصل الدم.

وكانتالنتائج كالآتي: زيادة ذات دلالة إحصائية معنوية في مستوى إنزيم بيتاجلكيورينيداز والخفاض ذو دلالة إحصائية معنوية بمستوى إنزيم كولينيستيريز في المرضى عنه في المجموعة الضابطة و ايضا كلما زادت شدة التسمم. كما تبين أن هناك علاقة مشتركة عكسية وذات دلالة إحصائية معنوية بين مستوى إنزيم كولينيستيريز ومدة المكوث بالمستشفى. بينما وجدت علاقة مشتركة طردية ذات دلالة إحصائية معنوية بين مستوى إنزيم بيتا جلكيورينيداز ومدة المكوث بالمستشفى. بالإضافة إلى وجود علاقة مشتركة عكسية وذات دلالة إحصائيةمعنوية بين مستوى إنزيم كولينيستيريز ومستوى إنزيم بيتا جلكيورينيداز سواء في المرضى او المجموعة الضابطة. البيانات المأخوذة من منحنى الروك أظهرت أن مستوى إنزيم بيتا جلكيورينيداز كان أكثر دقة (١٠٠%) في تشخيص الحالات عن مستوى إنزيم كولينيستيريز (٨٠%)، ولكن كلاهما كان له نفس نسبة الدقة في معرفة شدة التسمم أو في التنبؤ بنتائجه.

الاستنتاج: يستنتج من هذه الدراسة أن مستوى إنزيم بيتا جلكيورينيداز من الممكن أن يستخدم كعلامة حيوية في تشخيص و تقييم التسمم الحاد بالمبيدات المثبطة لإنزيم كولينيستيريز.

١ قسم الطب الشرعي والسموم الإكلينيكية – كلية الطب – جامعة الإسكندرية

٢ قسم الكيمياء الحيوية – كلية الطب – جامعة الإسكندرية

٣ طب الطوارئ – مستشفى العامرية