

The use of serial measurement of plasma cholinesterase in the management of acute poisoning with organophosphates and carbamates

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Abstract

Objective: To evaluate the benefits of using serial measurements of plasma cholinesterase (butyrylcholinesterase, BuChE) activity in the management of cholinesterase inhibitor insecticidal poisoning.

Method: After establishing and validating BuChE activity test, and making it available for clinical service in the toxicology laboratory at Jordan University Hospital. Serial measurements of BuChE were performed on samples taken from 10 symptomatic patients presented with the manifestations of poisoning due to acetylcholinesterase inhibitor insecticides during the year 2001. The number of serial repeats of BuChE activity tests ranged from 2 to 4 and from 8 to 11 for patients with carbamates and organophosphates (OPs) poisoning, respectively. The results of serial measurement of BuChE obtained from each patient's samples were used to draw a curve; three different types of curves were obtained from all patients samples.

Result: The obtained curves were found to follow our three proposed curves, which support our point view regarding the importance of the proposed curves in the differential diagnosis and treatment of cholinesterase inhibitor pesticides poisoning.

Conclusion: This study pointed out the importance of utilizing serial measurements of BuChE activity in the diagnosis and the management of organophosphates and carbamates poisoning. The BuChE activity results were used to support diagnostic and prognostic criteria that guided patient management and follow up. Applying those curves to large number of patients' samples will enhance its credibility. The study also demonstrated the importance of direct contact between toxicologist and physician in treatment of the pesticides poisoned patients.

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1. Introduction

Organophosphate insecticides are among the most toxic of all substances that cause poisoning in humans and are the most frequently encountered insecticides [1–5]. Organophosphate insecticides constituted more than 90% of pesticides mortality in Jordan [6]. High mortality rates resulted from intentional and accidental poisoning with organophosphates insecticides especially Parathion, leading to the implementation of strict regulation regarding their uses. In recent years, Methomyl carbamate is taking the lead as the most common insecticide used for agricultural and around the home in Jordan.

Organophosphate and carbamate insecticides are inhibitors to acetylcholinesterase, where the OPs irreversibly phosphorylate the enzyme while carbamates reversibly carbamylate the enzymes [7].

In clinical practice, diagnosis of AChE-inhibitor pesticides poisoning depends mainly on history of exposure, physical manifestations, clinical suspicion and laboratory support. The determination of the chemical identity of the pesticide substance that the patient was exposed to is ideal for antidotal management. Unfortunately this is an expensive and time-consuming laboratory procedure, and such a procedure is not available in most toxicology laboratories. Coye et al. [8] described the use of sequential plasma and BRC cholinesterase analyses in clinical confirmation of organophosphate poisoning. Others showed the benefit of measuring cholinesterase activity levels in predicting successful weaning of patient from

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mechanical ventilation [9], in severe OPs poisoning as well as improving the outcome [10]. However, a 23% variation in AChE levels exists among normal patients; therefore it is necessary to establish a baseline level to overcome the individual variation [11]. This practice is possible in occupational monitoring where the diagnosis of poisoning can be made by comparing post exposure AChE levels with baseline levels [11,12]. Such an approach cannot be accurately applied for patients presented with OPs poisoning utilizing a single AChE activity measurement. On the other hand, physicians usually request the butyrylcholinesterase (BuChE) activity level to confirm the diagnosis of symptomatic patients and aid management follow Aygun et al. [13].

The concept “serial measurements of BuChE-activity” in case of acute AChE-inhibitor pesticides poisoning was introduced in our toxicology laboratory in collaboration with the attending physicians. The concept was abstracted from our understanding the mechanism of action of either carbamates or Ops; the curves (A–C) in Fig. 1 were drawn according to our prediction of BuChE-activity levels behavior during the course of poisoning and treatment in the first 24 h. The result of the baseline sample usually shows a severe inhibition in the BuChE-activity level in case of acute poisoning of both carbamates and Ops. The serial measurements of BuChE-activity within the early hours of poisoning are expected to be a very valuable measure, which might be used in differential diagnosis of carbamates and Ops.

A significant increase in BuChE-activity is expected, if carbamates are the causative agent of poisoning. Curve (A) represents a reversible spontaneous cleavage of the carbamylated cholinesterase enzymes. While in the case of persistence inhibition in BuChE-activity poisoning with Ops are expected, and as a practice by physicians, patients were usually given one bolus dose of oximes antidote. The successive measurements of BuChE activity level are expected

to show a significant increase from the baseline level and remain high if the antidote dose is sufficient, and as a result there is no need for the administration of another dose of antidote. Curve (B) is expected to represents such cases. When the successive measurements of BuChE-activity level after the administration of the first bolus dose of antidote showed a sudden decrease in the level, a second bolus dose of the antidote might be required to increase the BuChE-activity level (enhance the breakage of the phosphorylated enzyme). Curve (C) is expected to represents such cases. Therefore, following the patterns of B and C curves could be very beneficial for the assessment of antidote efficacy.

The line which represents normal BuChE-activity level in Fig. 1 was constructed using blood samples taken from healthy subjects. In each case of acute AChE-inhibitor pesticides poisoning, patients' result (baseline) was compared with that of normal subjects during the study and as a policy in our toxicology laboratory to confirm poisoning with pesticides.

This work was designed to examine the proposed model curves (A–C) on actual data generated by the serial measurements of BuChE activity during the course of management of symptomatic patients presented with cholinesterase inhibitor insecticides poisoning, and to evaluate the benefits of utilizing direct communication between the toxicologist and the attending physician.

2. Material and methods

Patients presented to the hospital with symptomatic cholinesterase inhibitor insecticidal poisoning over a 1-year period starting from January 2001 were included in this study. Direct communication between toxicologist and duty physician was initiated at the time of admission to emergency room and during the course to treatment in the floor. Clinical manifestation at presentation, BuChE activity results and its interpretation, and the outlines of patient management and outcome were collected.

The proposed curves in Fig. 1 were examined on each case of poisoning to find out the possibility of utilizing it in the differential diagnosis of pesticides involved (Ops, carbamates) and to investigate the successfulness of oxime therapy as we proposed. The cooperation of the duty physician with toxicologist regarding the interpretation of BuChE-activity levels was very beneficial to the patients and to accomplish this work. The percentage of BuChE activity inhibition for each test was compared with the clinical data that was shown on the test request form or oral communication. For estimation of the percentage of BuChE activity depression, patients' samples were compared with plasma BuChE activity obtained from normal persons, which was considered as 100% activity. A total of 61 tests were conducted for both as part of the toxicological investigation for symptomatic patients presented with manifestation acetylcholinesterase inhibition and for their follow up monitor. As a protocol in our study, each case presented with symptomatic cholinesterase inhibitor insecticide poisoning, BuChE activity test was requested to be performed every 2–4 h during the first 24 h of administration to the hospital. The measurement of BuChE activity was conducted as described by others [14]. The procedure includes addition of plasma sample (20 μ l) and 0.1 ml of 5% solution of acetylthiocholine iodide to a 3 ml of dithiobisnitrobenzoic acid solution at 25 C. The mixture was mixed and the absorbance at 405 nm at 30 s interval was recorded for 2 min. The difference in absorbance was considered for estimation of BuChE activity. This test was established, validated and made available for the clinical toxicology laboratory service at JUH.

In drawing the proposed model curves in Fig. 1, 12 points (determinations) were used in constructing each curve, such an approach was followed in an attempt to produce meaningful curves only.

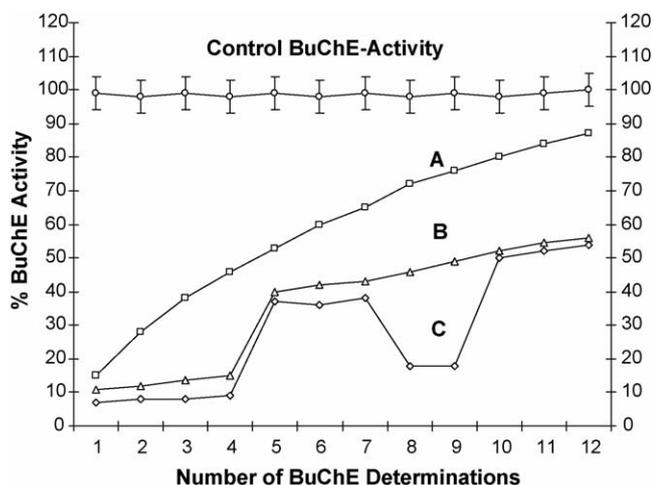


Fig. 1. Presents the proposed model curves which were tested on actual data generated by the serial measurements of BuChE activity, during the course of management of patients acutely poisoned with cholinesterase inhibitor insecticides. A; represents carbamate, B; represents Ops with single bolus dose of oxime and C; represents Ops with two bolus doses of oxime.

3. Results

A total of 61 BuChE activity tests were performed for 10 symptomatic patients that were presented to JUH over 1 year with the manifestations of acetylcholinesterase inhibitor insecticides poisoning. The coefficient of variation of the method used in measuring BuChE activity was 7%. Table 1 shows the number of cases, gender, age, type of insecticide, poisoning severity score (PSS), treatment, result of the first BuChE activity measurement, and number of BuChE tests performed for each case. The majority of poisoning cases was due to intentional oral ingestion of the insecticide (attempted suicide). The number of serial measurements of BuChE activity tests performed to diagnose, confirm, evaluate antidote efficacy, or to follow up the patients were 4 and 8–11 tests for patients presented with carbamates and OPs poisoning respectively. The identity of the specific agent was not available in most of the cases at the time of admission. The results of the serial measurements of BuChE-activity level for each patient (Table 1) were used in drawing a curve, the obtained curve was compared with the constructed proposed model curves (A–C) in Fig. 1. The curves patterns of actual cases were found to follow the proposed model curves. As a result, a tentative diagnosis was obtained.

Examples of the studied patients (Table 1) included a 29-years-old female (case no. 4). Presented to the emergency room with an attempted suicide with an unknown poison. Clinical manifestations included excessive and frothy salivation, dyspnea, cyanosis, wheezes, severe abdominal cramps and confusion PSS of 3. This presentation suggested the involvement of Ops or carbamate poisoning; 2 mg of atropine was given as a test dose followed by 2 mg every 10–20 min as required to keep the patient atropinized. A blood sample was taken and sent to the toxicology laboratory for BuChE measurement; a severe inhibition (10%) of the normal BuChE activity was noticed. Blood samples drawn after 2 and 4 h from the first sample showed persistence BuChE inhibition, which suggested an acute Ops poisoning, a bolus dose of oxime therapy was instituted. The measurements of BuChE activity after 2–4 h from oxime therapy showed a rapid and significant increase (30%) of the normal BuChE activity with improvement in clinical signs and symptoms. Such steady increase in

BuChE activity was suggestive of Ops poisoning which improved by oxime therapy. When the obtained BuChE activity results were drawn against the number of determination, the resulting curve was found to follow the proposed curve (A) model.

A 17-year-old female (case no. 5) was presented with difficulty of breathing, wheezes, lacrimation, abdominal pain and diarrhea (PSS 2) after the ingestion of Methomyl carbamate. The laboratory result showed a moderate inhibition of BuChE activity (22%) of the normal. Subsequent blood samples drawn after 2 and 4 h showed a significant increase in BuChE activity (36%) of the normal, which confirms the carbamate poisoning. This was also supported by the direct reversal of cholinergic manifestation by repeated administration of atropine.

4. Discussion

The majority of patients presented with serious clinical manifestation including severe respiratory difficulties due to both muscarinic and nicotinic stimulation and two patients had also CNS inhibition where one of these patients was transferred to (JUH) in deep coma and later was found to have brain death. The severity of poisoning of these patients were tabulated (Table 1) according to poisoning severity score, which ranged from 2 to 4. Patients with moderate presentation such as moderate bronchospasm, dyspnea, hypoxemia and confusion with cholinergic manifestation were given a PSS score of 2. A more severe form of these manifestations was given a PSS score of 3, while a PSS score of 4 was given for the patient presented with brain death. Poisoning severity score is a standardized scale for grading the severity of poisoning to allow qualitative evaluation of morbidity caused by poisoning and to provide better identification of real risks and comparability of data [15].

The standard treatment consists of reversal of the biochemical effects of acetylcholine with atropine for either OPs or carbamates poisoning and reactivation of the inhibited acetylcholinesterase with an oxime antidote, which should be administered as early as possible for OPs but it is contraindicated in carbamates poisoning [7]. Patients, who receive treatment usually recover from acute toxicity but may suffer from neurologic sequelae [16]. The management was started by

Table 1
Shows the number of cases, gender, age, type of insecticide, poisoning severity score, treatment, result of the first BuChE activity measurement and the number of BuChE tests performed for each case

Patient no.	Age	Sex	Poisoning severity score	Substance	Treatment	Lowest BuChE activity (%)	No. BuChE activity analysis
1	33	M	4	Carbamates	Atropine	25	4
2	52	M	3	OPs	Atropine and 2-pam	12	8
3	40	F	3	Carbamates	Atropine	18	4
4	29	F	3	OPs	Atropine and 2-pam	17	8
5	17	F	2	Carbamates	Atropine	32	4
6	22	M	3	Carbamates	Atropine	25	4
7	25	F	2	Carbamates	Atropine	30	4
8	3	M	3	Carbamates	Atropine	27	4
9	35	M	3	OPs	Atropine alone	22	11
10	26	M	4	OPs	Atropine alone	18	10

atropinization as is usually recommended. A direct and quick cooperation was introduced between the toxicologist and the attending physician in the diagnosis and the management of these patients utilizing serial BuChE activity measurements. The result of first BuChE activity measurement “referred as the baseline” level was considered as a reference for the consecutive serial BuChE activity measurements for each patient.

In the six cases of carbamate poisoning included in our study, a spontaneous improvement in BuChE activity upon the next consecutive measurement results (the second, third or fourth measurements) was noticed. The four BuChE measurements obtained for each case were found to follow the patterns of our proposed curve (A) model. At that point, the causative agent of poisoning was considered due to carbamates. In such cases, continued administration of the antidote atropine was recommended. One patient with severe carbamates poisoning (case 1) was transferred to the hospital 48 h post exposure died 8 h after admission in which brain death was reported.

While in the case of persistence depression of the BuChE activity in the early stage of poisoning, cases (2, 4, 9 and 10) two to three repeated measurements within 4–8 h from the baseline were performed with no significant improvement in BuChE activity level noticed, the sequential measurements of BuChE were found to follow the proposed model curves (B or C). At that point the causative agent of poisoning was considered due to OPs poisoning. Cholinesterase activators were instituted shortly after the confirmation of OPs exposure to obtain a good response as recommended by Thiermann et al. [16,22]. Such an approach was not applied to all OPs cases included in this study due to the shortage in the supply of antidote, and when it was available a single bolus dose (1 g/kg) of Pralidoxime was administered intravenously [7] for only two patients (cases 2 and 4), with continued administration of atropine after confirming the persistence of depression of BuChE activity (as proposed in curves B and C). The other two patients (9 and 10) were maintained atropinized for about 8 days until the patients regained spontaneous respiration and BuChE activity was more than 30% of the baseline. The name, amount and the concentration of OPs was not known, and since Pralidoxime was not administered to these cases and as a result they did not benefit from any further follow regarding the serial measurements of BuChE activity after the 8th day.

Once the acute OPs poisoning has been confirmed as suggested by the proposed model curves (B or C), the other important finding which might be obtained from using the model curves is the efficacy of antidotal therapy. In cases (2 and 4), and after 1–4 h of oxime therapy, BuChE activity showed a significant increase from the baseline. More samples were requested for the measurements of BuChE activity after the administration of antidote as recommended in the proposed model curves (B and C) in Fig. 1. A steady high level of BuChE activity results was noticed in case (4), which follow the patterns of curve (B). Such a steady high level of BuChE in case (4) showed the efficacy of oxime therapy. While in (case 2) a sudden drop in BuChE activity after oxime therapy was noticed 4–7 h, a second oxime therapy was recommended followed by

two repeats of BuChE-activity within 1–4 h. A steady and significant high BuChE level was observed, which suggests an effective antidotal therapy, this case showed similar patterns as in model curve (C). In the 10 symptomatic cases included in our study, BuChE activity in carbamates poisoned patients showed rapid recovery to normal levels (8–14 h) and the depression was less than that found in OPs poisoning which were 18–32 and 12–22% for carbamates and OPs poisoning, respectively.

Recently, Pralidoxime maintenance might be needed, as pointed out in the literature; where continuous Pralidoxime infusion along with aggressive atropinization after initial decontamination improved the outcome but not the duration of mechanical ventilation [17]. Others recommended the administration of Pralidoxime as a loading dose followed by a continuous infusion dose according to the severity of exposure; therefore permanent clinical improvement is achieved [18–21]. However, the effectiveness of oxime therapy in organophosphate poisoning is still a matter of debate [22] and the toxicokinetics and toxicodynamics of organophosphate poisoning vary not only with the route and extent of exposure, but also the chemical structure of the agent [9,23], and possibility of producing more active OP metabolites [7].

Worek et al. [19] evaluated *in vitro* studies with human erythrocyte AChE reaction parameters that contribute to the dynamic equilibrium of AChE inhibition, ageing and reactivation to help defining precisely the indications and limitations of oxime therapy in OPs poisoning. They reported that some OPs were characterized by slow spontaneous reactivation and low propensity for ageing. This kind of phosphorylated enzyme is particularly susceptible to reactivation by oximes. Also they reported that none of the oximes tested could be regarded as a universally suitable reactivator [19].

In conclusion, our study showed that a differential diagnosis of either carbamates or OPs poisoning can be achieved and the efficacy of antidote therapy can be evaluated by utilizing serial measurements of BuChE activities and the proposed model curves. Good clinical sense, in addition to a close observation of the clinical manifestation and respiratory management, would improve the outcome of their poisonings. It was also found that carbamates poisoning required fewer number of serial BuChE activity measurements to reach a diagnosis and to follow up the patient compared with that of OPs poisoning. Oxime antidotal therapy use by the attending physician should be reconsidered as recommended in recent literature. National and international scale studies addressing the use of our proposed model curves in the management of acute OPs and carbamates poisoning are worth to be taken in consideration by clinical and analytical toxicologist.

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