BUTYRYLCHOLINESTERASE ACTIVITY – BIOMARKER FOR PREDICTING THE OUTCOME IN ACUTE CHOLINESTERASE INHIBITOR POISONING – A 30-YEAR RETROSPECTIVE ANALYSIS

E.N. Gazzi¹, Victorita Ţorodoc¹, O. Petris¹, Liliana Taţau², Gabriela Dumitrescu¹, L. Ţorodoc¹, Cătălina E. Lupuşoru²
University of Medicine and Pharmacy “Grigore T. Popa” – Iasi
Faculty of Medicine
1. Department of Medical Specialties (I)
2. Department of Morpho-Functional Sciences

BUTYRYLCHOLINESTERASE ACTIVITY – BIOMARKER FOR PREDICTING THE OUTCOME IN ACUTE CHOLINESTERASE INHIBITOR POISONING – A 30-YEAR RETROSPECTIVE ANALYSIS (Abstract): Aim. To assess the role of butyrylcholinesterase (BuChE) activity as a predictive biomarker in acute cholinesterase inhibitor poisoning in a cohort from a regional tertiary care hospital. Material and methods: Plasma butyrylcholinesterase activity on admission and at regular intervals during admission and clinical outcomes of cases admitted to the Toxicology Clinic of "Sf. Spiridon" Emergency Hospital Iasi, Romania between 1983 and 2013 were evaluated. Results: A total number of 606 patients were included in the study. The mean BuChE-activity level on admission was 1.54 ml NaOH N/100. A correlation between the amount of ingested organophosphates/carbamates (OPs/CMs) and low cholinesterase activity on admission was found. 66.66% of the patients were admitted to hospital within 8 hours after poisoning. The initial, daily and mean total atropine doses administrated were 9.65 mg, 10.51 mg and 69.39 mg, respectively. 67.16% of the investigated patients received Toxogonin for 6.41 days showing a slow increase in BuChE activity afterwards. The average number of hospital days was 11.22. The study revealed that complications occurred in patients with BuChE-activity levels below 1.4 mL NaOH N/100. A positive correlation between mortality rate (3.8% of patients) and the lowest BuChE-activity level on admission (0.89 mL NaOH N/100) was found. Conclusions: BuChE activity on admission and its level during hospital stay represent an important predictive factor for acute cholinesterase inhibitors poisoning. Keywords: BUTYRYLCHOLINESTERASE, CHOLINESTERASE INHIBITORS POISONING, TOXICITY

Organophosphates (OPs) and carbamates (CMs) are toxic synthetic compounds that are used as: (a) insecticides, (b) chemical warfare agents, (c) therapeutic agents in veterinary and human medicine (1, 2, 3). Nowadays they are the most commonly used pesticides worldwide. The World Health Organization (WHO) estimates that every year over 3 million people experience acute poisoning by OPs, with more than 200.000 deaths. OPs self-poisoning is more common than unintentional intoxication, which is very rare, especially in areas where WHO class I toxicity pesticides are used (4). Farmers and people employed in agriculture are considered a
high-risk group of accidental intoxication with OPs (5, 6, 7). There are reports indicating that in many rural areas of Asia, where farmers are constantly using toxic pesticides, OPs are frequently used in suicides (8).

Their primary mechanism of action is the inhibition of esterase enzymes, especially acetylcholinesterase and butyrylcholinesterase. These two enzymes are biomarkers of OPs and CMs systemic toxicity. Acetylcholinesterase inhibition results in accumulation of acetylcholine and overstimulation of both muscarinic and nicotinic receptors. Red-cell acetyl-cholinesterase inhibition is correlated with poisoning severity and can guide the oxime and atropine therapy. Acute butyryl-cholinesterase inhibition does not seem to cause clinical features and is not related to severity of poisoning. It can be used as a marker of exposure to OPs or CMs and for measuring cholinesterase-inhibiting compound elimination from the body. Many OPs pesticides are more potent inhibitors of butyrylcholinesterase than they are of acetylcholinesterase (9, 10).

The main goal of this study was to evaluate BuChE activity as a biomarker of acute OPs/CMs poisoning, in patients admitted to the Internal Medicine and Toxicology Clinic of “Sf. Spiridon” Emergency Hospital Iasi between 1983 and 2013.

**MATERIAL AND METHODS**

We conducted a descriptive retrospective clinical study on patients with OPs/CMs poisoning admitted to the Internal Medicine and Toxicology Department of the Iasi "Sf. Spiridon" Emergency Hospital aimed at evaluating the phenomenon of acute cholinesterase inhibitor poisoning over a period of 30 years.

Hospital records of patients diagnosed with acute accidental/voluntary OP or CM poisoning between the years 1983-2013 were reviewed.

The obtained data were used to create a complex and complete medical profile for each intoxicated patient.

A total of 53 parameters were assessed. The data on BuChE activity were evaluated by the analysis of following parameters: (a) BuChE-activity level on admission; (b) BuChE activity during hospital stay (c) time to serum cholinesterase return to normal physiological levels.

BuChE activity on admission was expressed as ml of NaOH N/100, not as mU/mL due to the fact that in most patients the Michel method for BuChE determination was used. Reference values were considered between 4-6 mL NaOH N/100. The others patients were assessed using the Ellman method; the obtained cholinesterase levels were expressed as mU/mL and then converted to mL NaOH N/100.

The experimental protocol of this investigation was implemented according to recommendations of the University Committee for Research and Ethical Issues, and to the guidelines stipulated in the EU Charter of Fundamental Rights, regarding clinical investigations on human subjects (10).

Statistical analysis of data was performed using SPSS for Windows version 19.0 and one-way ANOVA method, followed by Bonferroni post hoc test. Chi-square test was used for comparing means and variables and frequency analysis, considering the level of significance for p value less than 0.05.

**RESULTS AND DISCUSSION**

A total number of 606 patients were included, accounting for 11% of the total number of acute poisonings. The highest number of cholinesterase inhibitor poison-
Butyrylcholinesterase activity – biomarker for predicting the outcome in acute cholinesterase inhibitor poisoning – a 30-year retrospective analysis

BuChE activities on admission and during hospital stay were assessed using several parameters: (a) means of patient transportation to hospital (ambulance or means of transportation others than ambulance); (b) time interval between poisoning and hospital admission; (c) amount of ingested OPs; (d) age and sex; (e) clinical parameters; (f) treatment (initial, daily and total dose of atropine, Toxogonin® treatment); (g) time to return to normal BuChE levels; (h) course; (i) complications; (j) discharge status.

On admission most patients had a BuChE level around 1.5 mL NaOH N/100 (fig. 1). We found a correlation between the amount of ingested OPs/CMs and low cholinesterase activity on admission (p=0.002). More than half of the patients (58.74%) had ingested a small amount of OPs/CMs (average 69.19 mL). In this group, the cholinesterase activity level on admission was on average 1.44 mL NaOH N/100, with a slow increasing tendency. A rapid increase in cholinesterase activity was found in patients that ingested a higher amount of OPs/CMs (84.73 mL). In these patients, the cholinesterase activity on admission was 2.33 mL NaOH N/100 (fig. 1).

In patients older than 80 years, the average BuChE level was 0.22 mL NaOH

Fig. 1. Cholinesterase activity on admission. Correlations between butyrylcholinesterase activity on admission and hospital addressing time.

The estimated average time for admission to the emergency department after poisoning was 8.8 hours. 66.66% of the patients admitted to hospital within 8 hours after poisoning were using personal means of transportation and had an average serum BuChE level of 1.5 mL NaOH N/100. 6.72% of the patients admitted within 16 hours after poisoning were transported to hospital by ambulance and had an average serum BuChE level of 0.5 mL NaOH N/100 (fig. 1).

It is worth mentioning that patients using personal means of transportation reached the hospital in a shorter time frame than those that transported by the ambulance.
The highest average BuChE level recorded was 1.88 mL NaOH N/100 in patients aged 16 to 19 years. There were no statistically significant differences (p=0.084) in average BuChE levels on admission between females and males.

The higher the average BuChE level on admission, the less complicated were the clinical manifestations of poisoning (p=0.000).

The initial, daily and total mean atropine doses administrated were 9.65 mg, 10.51 mg and 69.39 mg, respectively. Patients with average BuChE levels around 2.5 mL NaOH N/100 and 0.5 mL NaOH N/100 on admission were given a lower atropine dose. In patients with a plateau BuChE level on admission followed by an increase during hospital stay, the highest total atropine dose - 90.52 mg was administrated. The smallest total atropine dose was administrated in patients that had a dramatic increase in BuChE activity (fig. 2).

The initial and daily atropine doses were almost similar. There were no significant differences between initial and daily doses of atropine when compared with BuChE activity on admission. One significant difference was found between the total dose of atropine and BuChE activity on admission: the lower BuChE activity on admission, the higher the atropine dose administrated to the patient.

![Graph](image)

**Fig. 2.** Correlations between butyrylcholinesterase activity on admission, atropine and Toxogonin therapy.

The mean duration of Toxogonin treatment was 5.74 days. 67.16% of the patients (BuChE on admission 1.44 mL NaOH N/100) received Toxogonin for 6.41 days and showed a slow increase in BuChE activity afterwards. The longest duration of
Butyrylcholinesterase activity – biomarker for predicting the outcome in acute cholinesterase inhibitor poisoning – a 30-year retrospective analysis

Toxogonin treatment (7.23 days) was in patients with a decreasing BuChE tendency after admission, followed by an increase in BuChE activity. The shortest Toxogonin treatment (2.74 days) was given to the patients who had a dramatic increase in BuChE activity after admission (fig. 2).

No correlation was found between the total number of Toxogonin treatment days and the initial BuChE level on admission. There was a strong correlation between the total number of Toxogonin treatment days and the further BuChE activity during hospital stay (p=0.000).

Of all patients, 9.75% received exogenous cholinesterase. There were no statistically significant differences regarding BuChE initial activity between these patients and the patients who did not receive exogenous cholinesterase (p=0.361).

The following correlations were found: BuChE activity increased in patients with the highest BuChE levels on admission; BuChE activity was oscillating in patients with the lowest BuChE levels on admission; the return to normal of serum cholinesterase activity was positively correlated with the mean initial BuChE level on admission.

In most of the patients (67.73%) cholinesterase activity returned to normal within 6-7 days after admission, following an ascending slope (fig. 3). In patients with the lowest initial BuChE level on admission (0.5 mL NaOH N/100) cholinesterase activity was oscillating, described by alternated ascending and descending slopes, and was not normal at the time of the discharge from hospital. In patients with the highest mean BuChE level on admission (2.59 mL NaOH N/100) cholinesterase activity had rapidly increased, returning to normal physiological values 3 days after admission (fig. 3, 4).

**Fig. 3.** Correlations between the course of butyrylcholinesterase activity, frequency and time to normalization.
Fig. 4. Correlations between mean BuChE activity on admission, its dynamics and patient course

The higher the BuChE on admission, the better the patient clinical course - p=0.000.

Of the total number of patients, in 67.73%, with a slow increasing tendency for BuChE, the average length of hospital stay of 11.65 days. In patients with a dramatic BuChE increasing tendency, the average length of the hospital stay was 6.7 days. The highest average length of stay was 13.95 days and was recorded in patients with a plateau-like BuChE activity followed by an increase in activity – mean BuChE level on admission 1.44 mL NaOH N/100. The mean length of hospital stay was positively correlated with BuChE course and its normalization time.

Our investigation demonstrated a higher incidence of complications in patients with BuChE levels below 1.4 mL NaOH N/100. In patients with BuChE levels above 1.66 mL NaOH N/100 (73% of patients) no complications were present.

The most frequently recorded complications were acute pulmonary edema (10.6% of cases), in patients with of 1.29 mL NaOH N/100 and toxic myocarditis (3.2% of cases), in patients with a mean BuChE activity of 1.38 mL NaOH N/100 on admission (Fig. 5). A BuChE level of 0.85 mL NaOH N/100 on admission was associated with such complications as: respiratory problems (1.3% of cases) and cardiac rhythm disturbances (0.7% of cases).

Our results indicate a positive correlation (p=0.072) between the presence of both cardiac arrest (3% of cases) and sudden death (0.3% of cases), and the mean level of BuChE activity (1.2 mL NaOH N/100).

A positive correlation was also found between mortality (3.8% of patients) and the lowest average BuChE activity on admission - 0.89 mL NaOH N/100.

Of the total number of patients, 80% were discharged at the recommendation of the treating physician (mean BuChE activity level of 1.63 mL NaOH N/100 on admission), while 10.41% on request (1.13 mL NaOH N/100) (fig. 5).
CONCLUSIONS

In our study BuChE activity on admission and its course during hospital stay represents an important prognostic factor for cholinesterase inhibitors acute poisoning. The decrease of BuChE activity was positively correlated with the degree of poisoning. A tendency of BuChE activity to increase in patients with high BuChE levels and an oscillating course in patients with low BuChE activity level on admission were found. Complications were reported in patients with BuChE levels below 1.4 mL NaOH N/100, but not in patients with BuChE levels above 1.66 mL NaOH N/100. The incidence of mortality was correlated with low mean BuChE levels on admission.
ACKNOWLEDGEMENT

This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/136893.

REFERENCES


NEWS

BACTERIAL INFECTION - SIDE EFFECTS OF COSMETIC TREATMENT

The lip augmentation and wrinkle smoothening are very commune, but the cosmetic treatment which consists in injection of soft tissue fillers can induce tender subcutaneous lumps and lesions difficult to treat. A recent study published in the journal Pathogens and Disease, showed that side effects of filler treatment are caused by bacterial infection. Using a mouse model, the researchers evaluated capacity of fillers for sustaining bacterial infections and the possible treatment strategies. Three type of gel contaminated with Pseudomonas aeruginosa, Staphylococcus epidermidis, and Propionibacterium acnes were injected in a mouse model. The results showed that the permanent gel was able to sustain bacterial infection more than the semi-permanent gel. Temporary gel did not cause infection. Also, the study revealed that antibiotic treatment become inefficient if bacteria have formed a biofilm. This research emphasizes importance of antibiotics injection together with the filler itself, for prevent bacterial complications induced by cosmetic treatment (Alhede M, Er Ö, Eickhardt S et al. Bacterial biofilm formation and treatment in soft tissue fillers. Pathogens and Disease. 2014; DOI: 10.1111/2049-632X.12139).

Luncă Cătălina