Study of Acute Anticholinesterase Poisoning Cases and the Predictive Relevance of Caspase Markers

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Abstract

Objective: To assess the pattern and outcome of acute anticholinesterase poisoning cases admitted to Menoufia Poison and Control Center (MPCC) over 1 year and to explore the potential role of caspase markers in predicting the outcomes.

Background: Acute anticholinesterase poisoning is a frequent cause of morbidity and mortality. It causes mitochondrial dysfunction, increased reactive oxygen species production, and lipid peroxidation. This increases caspase biomarker activity.

Patients and methods: A prospective cohort study was done at (MPCC) over one year from (the beginning of November 2020) to (the end of October 2021). Acute anticholinesterase intoxication cases admitted to MPCC were studied. Both socio-demographic and clinical data were gathered from each patient and recorded in a clinical toxicological sheet. The cases were categorized according to peradynia organophosphorus poisoning score. Caspases 3 and 9 were measured before and after treatment.

Results: This study included 68 patients. The commonest age was (20-< 30 y). Females outnumbered males (58.83% vs. 41.17%). Regarding POP score, (45.59%) of cases were moderate. The mortality rate constituted (8.83%). Caspase level increased before treatment and decreased after treatment. The specificity of Caspase 3 and caspase 9 to predict mortality was (90.9 and 93.9%, respectively), while the sensitivity of both was (83.3%).

Conclusion: Acute anticholinesterase poisoning makes up a significant portion of all cases treated at MPCC. Caspase 3 and 9 levels increased significantly after acute anticholinesterase poisoning. Caspase markers have an important role in predicting mortality in these cases.

Keywords: Anticholinesterase, Caspases, Morbidity, Pesticides, Sensitivity

1. Introduction

In Egypt and developing countries, anticholinesterase compounds are among the most often utilized chemical substances due to their availability and effectiveness. The WHO estimates that there are 3 million cases of pesticide poisoning each year and up to 220 000 deaths in developing countries [1]. There is an annual increase in the number of acute anticholinesterase poisoning cases in Egypt whether suicidal or accidental [2].

These compounds act by inhibiting acetylcholine esterase enzyme causing overstimulation of acetylcholine receptors. The clinical manifestations of poisoning are mainly due to muscarinic, nicotinic, and central nervous system (CNS) receptors overstimulation. Acute anticholinesterase poisoning is associated with oxidative stress, lipid peroxidation, low glutathione level, and damage to cell membranes resulting in cell death and adverse effects on multiple systems in the body [3]. Routes of exposure involve inhalation, ingestion, or dermal contact. Peradynia organophosphorus poisoning scale was proposed for evaluating the severity of acute anticholinesterase poisoning cases [4].

Diagnosis of acute anticholinesterase poisoning is based on the history of exposure, the presence of symptoms and signs, and the decrease in serum pseudocholinesterase enzyme levels [5].

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2. Patients and methods

Caspases are a family of endoproteases that cut proteins at specific sequences and play essential roles in inflammation and apoptosis. The members of the caspase family are classified as inflammatory or apoptotic. The caspases -1, 4, -5, and -11 are grouped as inflammatory caspases and the caspases -2, -3, -6, -7, -8, -9, and -10 are grouped as apoptotic caspases. These apoptotic caspases are further subdivided into two groups: the initiator caspases (caspase-2, -8, -9, and -10), which are the first to be activated in response to a signal, and the executioner or effector caspases (caspase-3, -6, and -7) that carry out apoptosis and cell death [6].

Anticholinesterase compounds directly interact with cellular membranes causing disturbances in their structure, mitochondrial dysfunction, enhanced production of reactive oxygen species (ROS), and subsequent lipid peroxidation. Lipid peroxidation is accompanied by an alteration of the antioxidant defense system in different organs. The increase in the activity of caspase biomarkers after acute exposure to anticholinesterase compounds may be due to oxidative stress through releasing of cytochrome C that activates them to induce apoptosis and cell death [7].

The cause of death in acute anticholinesterase poisoning cases is usually due to respiratory failure, but may also be due to other complications such as arrhythmia, pneumonia, pulmonary edema, pancreatitis, and renal failure [8].

Hence, this study aimed to assess the pattern and outcome of acute anticholinesterase poisoning cases admitted to Menoufia Poison and Control Center (MPCC) over one year, as well as to explore the potential role of biochemical caspase markers in predicting the outcomes.

2. Patients and methods

After receiving ethical approval from the Menoufia University Faculty of Medicine's ethical committee NO (11/2020FOR-35), this study was conducted on acutely anticholinesterase-poisoned patients who were admitted to MPCC over one year, from (the beginning of November 2020) to (the end of October 2021). After obtaining written, valid consent from the participants or their legal guardians, the study included patients of both genders and from various age groups. Patients who received treatment before presenting to our hospital, patients who gave a history of co-ingestion, and patients who refused to give consent or participate in the study were excluded from this study. A detailed history was taken regarding sociodemographic data (age, sex, residence, marital status, and occupation). Also, clinical manifestations, classification of cases according to peradynia organophosphorus poisoning score, and outcomes were included. POP score was used to assess the severity. It includes a group of clinical parameters such as (level of consciousness, seizure activity, pulse rate, respiratory rate, pupil size, and fasciculations). Cases were graded as mild (score 0–3), moderate (score 4–7), and severe (score 8–11) poisoning.

Laboratory investigations (such as ABG, complete blood count, serum electrolytes, liver and kidney function tests) and radiological investigations (such as chest radiography or CT chest) were done when needed. Specific toxicological investigations (as thin layer chromatography, and serum Pseudocholinesterase levels by Single beam spectrophotometer (T60 UV VIS Spectrophotometer UK) were done.

For measurement of caspase marker levels; blood samples were collected from anticholinesterase-poisoned patients twice, the first immediately after admission to the hospital (before starting treatment by atropine and oximes), and the other after treatment and full atropinization of the patients (as dryness of secretions, dilated pupils, warm dry flushed skin and increased heart rate). Also, blood samples were collected from ten healthy volunteers who served as control, were clinically free, and were not exposed to anticholinesterase compounds.

Caspase markers 3 and 9 activity was estimated by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (BlueGene Biotech; Shanghai, China).

Statistical analysis: All Statistical tests were conducted using the R software version 4.1.2 (R Foundation for Statistical Computing, Vienna, Austria). The following statistics were used: Analytical statistics include the $\chi^2$ test and the Fisher Exact test for descriptive statistics, respectively. $P$ value greater than 0.05 was deemed to be statistically insignificant. Statistical significance was defined as a $P$ value of 0.05. Statistically highly significant was regarded to be a $P$ value less than 0.001 [9].

3. Results

This study comprised sixty-eight acute anticholinesterase poisoning patients who were admitted to the MPCC between November 2020 and October 2021 and who met the inclusion criteria.

According to the age distribution, the most affected age group was (21–30) years old which represented (42.65%), while the age group under 10 years old was the least. The proportion of females was (58.83%) compared with (41.17%) of males. The majority (58.83%) of cases came from rural regions.
while the remaining portion (41.17%) came from urban areas.

Regarding marital status, (54.41%) of cases were married, followed by single cases (39.71%), widows, and divorced cases (2.94%) for each. It was noticed that the most affected cases were illiterate (32.35%), followed by elementary education (23.53%) followed by secondary education (17.65%), high school education (14.71%), then college (11.76%). As regards occupation, students represented (47.06%) of the cases, then farmers (27.94%), followed by unemployed (8.82%), and lastly housewives (4.41%).

Additionally, 10 healthy volunteers served as controls, (30%) of them at the age group (20–30 year) and (30%) in the age group (30–40 year). The majority of them were female and from rural areas. Also, (50%) of them were single and the largest number were at college. (Table 1).

The relation between outcome and mode of poisoning was statistically significant ($P$ value < 0.05), as (50%) of survived cases were accidental while all dead cases were suicidal. Also, there was a significant relation between the outcome of cases and time passed since poison exposure ($P$ value < 0.05), (48.39%) the survived cases presented to the hospital within 2 h from poisoning, while (66.67%) of dead cases presented after 8 h. A significant relationship was found ($P$ value < 0.05) between the severity grades and case outcomes according to the Peradeniya organophosphorus poisoning (POP) scale. A 50% of the surviving cases were moderate, (36.71%) were severe, and (11.29%) were mild, although all of the dead cases were severe.

According to TLC, about (62.9%) of survived cases were positive organophosphate, (37.1%) of survived cases were positive carbamate and all dead cases were positive organophosphate.

In addition to that, the relation between the place of admission and the outcome of cases was highly significant. There were 6 dead cases, all of them were admitted to the ICU while (74.19%) of the lived cases were admitted to the department. The relation between the duration of hospital stay and the outcome of cases was highly significant, $P$ value (<0.001). About (83.33%) of dead cases were admitted more than 7 days, on the other hand about (48.38%) of the lived cases were admitted from 3 to 7 days and (33.87%) were admitted from 1 to 3 days.

The relation between the outcome of the studied cases and caspase 3 level before treatment was highly significant where the mean level and standard deviation in the dead cases was (6.27 ± 1.31 ng/ml) versus (3.2 ± 1.6 ng/ml) in the survived cases with $P$ value (<0.001). Also, there was a highly significant relation between the caspase 9 levels of cases before treatment and outcome, where the mean of the dead cases was (6.21 ± 1.28 ng/ml) versus (3.41 ± 1.56 ng/ml) in the survived cases with $P$ value (<0.001) (Table 2).

The serum activity of caspase 3 of acute anticholinesterase poisoning cases before treatment was significantly increased as compared with control groups ($P$ value < 0.001). Also, a highly significant relation between control and cases after treatment and between cases before and after treatment ($P$ value < 0.001).

The mean value of serum caspases 3 of the controls was (0.45 ± 0.23). The mean value of serum caspases 3 of cases before starting treatment was (3.67 ± 1.91) and after treatment was (1.12 ± 0.90) Fig. 1a.

The difference in caspase 9 activity was statistically highly significant between control and cases before treatment and highly significant between control and cases after treatment ($P$ value < 0.001). In addition to that, there was a statistically highly significant relation in caspase 9 activity between cases before and after treatment.

| Table 1. The distribution of acute anticholinesterase toxicity cases regarding sociodemographic data (No = 68). |
|-------------------------------------------------|-------------------------------------------------|
| Sociodemographic characteristics | Studied group $N = 68 \ n (%)$ | Control $N = 10 \ n (%)$ |
| Age: | | |
| <10 y | 2 (2.94) | 0 |
| 10–<20 y | 13 (19.11) | 2 (20) |
| 20–<30 y | 29 (42.65) | 3 (30) |
| 30–<40 y | 15 (22.06) | 3 (30) |
| 40–<50 y | 5 (7.35) | 1 (10) |
| 50–<60 y | 4 (5.88) | 1 (10) |
| Sex | | |
| Female | 40 (58.83) | 7 (70) |
| Male | 28 (41.17) | 3 (30) |
| Residence | | |
| Rural | 40 (58.83) | 8 (80) |
| Urban | 28 (41.17) | 2 (20) |
| Marital status | | |
| Single | 27 (39.71) | 5 (50) |
| Married | 37 (54.41) | 3 (30) |
| Divorced | 2 (2.94) | 1 (10) |
| Widow | 2 (2.94) | 1 (10) |
| Education | | |
| Illiterate | 22 (32.35) | 1 (10) |
| Elementary | 16 (23.53) | 1 (10) |
| Secondary education | 12 (17.65) | 0 |
| High school | 10 (14.71) | 1 (10) |
| College | 8 (11.76) | 7 (70) |
| Occupation | | |
| Employee | 8 (11.76) | 2 (20) |
| Farmer | 19 (27.94) | 0 |
| Housewife | 3 (4.41) | 2 (20) |
| Student | 32 (47.06) | 4 (40) |
| Unemployed | 6 (8.82) | 2 (20) |
The mean value of serum caspases 9 of controls was \((0.42 \pm 0.23)\). The mean value of serum caspases 9 of cases before starting treatment was \((3.84 \pm 1.82)\) and after treatment was \((1.26 \pm 0.87)\).

The cut-off value of the caspase 3 activity was \((6.24)\) ng/ml. The caspase 3 activity showed an area under the curve (AUC) of \((0.90)\) (The accuracy of the test is excellent) with a positive predictive value of \((0.714)\), sensitivity of \((83.3\%)\), and specificity of \((93.9\%)\) (Table 3).

4. Discussion

Acute anticholinesterase poisoning is considered one of the most frequent and most common causes of human toxicity globally because of its low cost and easy availability, it leads to severe manifestations and death. There is annual increase in the number of acute anticholinesterase-poisoned cases in Egypt [10]. This might be related to the widespread usage of these toxic compounds, their application in agricultural and environmental pest
Fig. 1. Box plot chart shows A: Caspases 3 activity of controls and acute anticholinesterase poisoned cases before and after treatment. B: Caspases 9 activity of controls and anticholinesterase compounds poisoning cases before and after treatment.
control, and lack of the patient awareness about the danger of acute toxicity by these compounds [2].

This study included 68 patients of acute anticholinesterase poisoning who met the inclusion criteria and were admitted to MPCC over one year, from the beginning of November 2020 to the end of October 2021. Poisoned cases aged (20–30 year) were predominant (42.65%) followed by those aged 30–40 (22.06%), while the lowest percentage was below 10 years old (2.94%). This typically agreed with Kumar et al. [11] who noticed that most of the patients were in the age group between 21 and 30 years. The high rate of poisoning among adults may be due to failure in romantic relationships, failure in exams, and problems adjusting to life with their parents or peers. According to the annual report of Poison Control Center, Ain Shams University Hospitals in 2011, adults in Egypt had the highest rate of poisoning due to the extreme emotional and financial difficulties that faced adult age groups [12].

As regards sex, females outnumbered males (58.83% vs. 41.17%). This result was similar to that reported in a study by Tallat et al. [13] who reported a higher incidence in the number and percentage of female patients. This may be explained by the increased frequency of domestic violence, family conflicts, and emotional liability in women, which can result in psychological stress.

Residence-based evaluation showed that the percentage of cases from rural areas (58.83%) is higher than that from urban areas (41.17%). Menoufia is a well-known agricultural region where anticholinesterase chemicals are used freely and extensively in farming. The rising incidence of poisoning in rural regions may be attributed to these elements as well as people’s carelessness, challenging lives, and ignorance. Oreby and El-Madah [14] reached the same result.

Regarding marital status, the majority of cases in this work (54.41%) were married, while (39.71%) were single. This coincided with Hodeib and Khalfia [15]. Who reported that married patients were more than unmarried. The common risk factors explaining that were problems at home, in society, abuse of drugs or alcohol, and marital issues. Presence of peer pressure, financial disparity, lack of opportunities, and family problems among married people [4].

In light of our study’s findings, it was observed that the most affected cases were illiterate who constituted (32.35%) and the lowest percent were highly educated (11.76%). This coincided with Shrestha et al. [16] who reported in their study that most of the cases were not educated. Illiteracy causes negative thoughts towards life and lack of education leads to an inability to solve problems [17]. In the present research, most patients were students (47.06%) followed by farmers (27.94%) and the least were housewives (4.41). A similar observation was found by Thapa et al. [17]. Students are becoming more vulnerable to acute poisoning as a result of academic issues like failing in exams or falling in love stories and inability to solve their problems because of immature thinking.

Oral ingestion was the most common route of acute exposure. About (85.48%) of survived cases and (83.33%) of dead cases of acute anticholinesterase poisoning were through ingestion. Sinha et al. [18] were in agreement with our study when noticed that the majority of acute organophosphorus poison cases were through oral route exposure rather than inhalation or dermal route exposure. Oral ingestion is identified as the first route of acute poisoning. This might be due to the ease of administering the toxic agents orally as compared with other routes.

According to the mode of poisoning, the results of the current study showed a statistically significant relation between outcome and mode of poisoning (P value ≤ 0.05) as (50%) of survived cases were accidental and (50%) were suicidal whereas all dead cases were suicidal. This could be due to a stressful atmosphere whatever the source and type of the stress, it may be financial, marital, employment, or unemployment stress. This agreed with Hiremath et al. [19] who stated that the suicidal mode of poisoning was the commonest among the studied cases.

In the current work, the majority of cases were exposed to the poison in a liquid form, while the rest of the cases were exposed to it in a powder form. Amir et al. [4] agreed with our study when reported that most of the patients ingested the insecticides in
a liquid form. This can be explained by that liquid forms are widely available and easier to be ingested.

The prognosis and outcome of the cases depend greatly on the amount of time that passes between ingesting the poison and arriving at the hospital. In this work, the relation between the outcome of cases and time passed since poison exposure was significant (P value < 0.05). Most of the survived cases (48.39%) presented to the hospital within 2 h from poisoning, and (66.67%) of dead cases presented after 8 h. A high cure rate is highly correlated with early arrival at the hospital and obtaining medical care. Rapid patient transfer to the MPCC might be due to its close vicinity to the surrounding rural areas, which represented the majority of the poisoning cases.

A similar result was noticed by other researchers Anjana and Neeta [20], who stated in their study that most of the patients presented to the hospital within the first 2 h after acute exposure to the poison. The time interval between intake of the poison and hospital admission is essential because early management reduces morbidity and mortality. So, higher mortality rates are typically associated with patient ignorance of the significance of getting medical care as soon as possible.

Acute anticholinesterase poisoning patients were classified by using the POP scale, which uses clinical parameters to represent the muscarinic, nicotinic, and central effects of these compounds. There was a significant relation between the outcome of cases and grades of severity according to POP score (P value < 0.05). All dead cases were severe and more than half of the surviving cases were mild to moderate as per the POP scale. Similar results were given by Eisa et al. [21] at Zagazig University hospitals when reported that the largest number of survived cases was moderate (60.1%). POP scale can be used for all kinds of patients as it does not need patient consent [4]. Thus, the POP scale is an easy-to-use, reasonably priced method for predicting mortality.

Considering the duration of hospital stay, the relation between the duration of hospital stay and the outcome of cases was highly significant (P value ≤ 0.001). About (83.33%) of dead cases were admitted to hospital for more than 7 days, on the other hand, the majority of the lived cases were admitted from 1 to 3 days or from 3 to 7 days early presentation of most cases in this study, early diagnosis, and appropriate management may account for the short length of hospital stay.

The same result was revealed by Shah et al. [22] who stated in their study that the majority of survived patients stayed in the hospital for 3–7 days. Furthermore, our study and the study of El-Bialy et al. [23] supported the idea that patient mortality rates were lowered when hospital stays were shorter.

As regards place of admission, there was a highly significant value between the place of admission and the outcome of cases, (P value ≤ 0.001). There were 6 dead cases, all of them were admitted to the ICU as they were mechanically ventilated or developed secondary complications while (74.19%) of the lived cases were admitted to the department.

The present study revealed that serum caspase 3 and caspase 9 activity was significantly increased in acute anticholinesterase poisoning cases before treatment as compared with control groups and decreased after treatment (P value < 0.001). Also, there was a highly significant relation between the outcome of acute anticholinesterase poisoned cases and caspases 3 and caspases 9 levels before treatment, P value (<0.001) whereas the dead cases had higher levels of caspase 3 and 9 than the cases that survived. Oxidative stress can account for the increase in caspase markers 3 and 9 levels following acute exposure since it generates cytochrome C and activates caspases, which cause cell death and apoptosis [7].

Caspase 9 and caspase 3 are activated by the primary intrinsic pathway, which is initiated by the release of cytochrome C. The death receptor initiates the extrinsic route at the cell surface, which is then followed by the activation of caspase 3. It is believed that caspase 3 is an effector caspase while caspase 9 is one of the primary initiators caspases. The two mechanisms combine to produce apoptotic changes in cells, including mitochondrial malfunction, disrupted mitochondria-dependent processes, increased production of ROS, and lipid peroxidation [24]. After administration of the treatment, the activity of caspases decreases or returns to normal due to inhibition of oxidative stress and stoppage of apoptosis. The same results were given by Eldesoqui et al. [25] who reported in their study that malathion-receiving rats showed a significantly increased caspase-3 activity in the renal tubular cells when compared with the control group, on the other hand when malathion-intoxicated rats treated with curcumin as an antioxidant, they showed a significant reduction of caspases 3 level due to improvement in the cellular antioxidant status. Furthermore, Owumi and Dim [6]. Reported in their study that there was a marked increase of caspase-3 activity in the hepatic and renal cells of rats when exposed to the insecticide chlorpyrifos and this resulted in apoptotic cell death. Also, they observed that the caspase-3 activity was reduced in rats that were exposed to chlorpyrifos and treatment.
In line with this study, Zhang et al. [7] found that caspase 9 and 3 activity increased during acute pesticide exposure and before treatment, they coincided with the current findings. In agreement with the current finding, Ojha et al. [26] reported that rat cells exposed to pesticides in vitro showed a substantial increase in caspase-3 and caspase-9 activity. El-Bialy et al. [23] also stated that acute pesticide poisoning in rats was associated with the production of ROS, which triggered apoptotic cell death and caspase-3 expression in both renal and hepatic cells.

Also, Hussain et al. [27] noticed in their study that treatment of rats’ hepatic cells with the insecticide (pyrethroid) increased the expression of caspases 3 and 9 when compared with the control group. On the other hand, after supplementation of these cells with antioxidants the level of these markers was lowered. Bahar and Eraslan [28] noticed in their study that caspase 3 and caspase 9 levels significantly increased in the liver of albino rats on acute exposure to a carbamate insecticide when compared with the control group.

The present study revealed that the caspase 3 activity showed an AUC of (0.907); this indicates that the accuracy of the test is excellent with a sensitivity of (83.3%) and specificity of (90.9%). Caspase 9 showed an AUC of (0.899) this means that the accuracy of the test is good with a sensitivity of (83.3%) and specificity of (93.9%). AUC is commonly used to assess the accuracy of the tests [29]. This suggests that caspase 3 is more accurate than caspase 9 in predicting mortality of acute anticholinesterase-poisoned cases. Besides that when the cut-off point of caspase 3 levels greater than or equal to 6.24 and of caspase 9 levels greater than or equal to 6.41, this indicates that these cases will have a bad prognosis. A similar result was reported by Tallat et al. [13].

Unlike our study, Abdelhamid et al. [30] reported in their study that caspase-3 and caspase-9 were poor mortality indicators when used as predictive biomarkers in acute aluminum phosphide poisoning as they had an AUC of 0.64 and 0.61, respectively, (accuracy of the test is poor when AUC of (0.60 – 0.70)).

4.1. Conclusion

Acute anticholinesterase toxicity is considered a medical emergency that requires immediate management. The most affected age group was (21 – 30) years old. Oral exposure was the most common route of acute poisoning. The POP scale is effective in assessing severity in patients with acute anticholinesterase compound poisoning. The serum level of caspase biomarkers 3 and 9 was a reliable biomarker for predicting mortality in these cases as high blood levels of caspase markers were associated with poor prognosis. Caspase 3 is considered more accurate than caspase 9. It is recommended to estimate the caspase markers 3, 9 levels to predict the prognosis of these cases.

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Ethics information

There was ethical approval from Menoufia University, Faculty of Medicine Ethical Committee (No. 11/2020FOR-35).

Conflict of interest

There are no conflicts of interest.

References


