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Oxidative Stress Parameters, Can They Predict Mortality in Acute Aluminum Phosphide Poisoning?

Shymaa M. N. Abdulrhman*, Raghda H. Deraz, Laila M. E. Sabik
Department of Forensic Medicine and Clinical Toxicology,
Faculty of Medicine, Zagazig University, Egypt
***Corresponding author:** Shymaa M. N. Abdulrhman, **Mobile:** (+20) 01140292959, **Email:** shymaanasrallah93@gmail.com

ABSTRACT

Background: Aluminum phosphide (AlP) is used excessively in Egypt as a fumigant and preservative for the grains. Recently, the suicidal accidents due to AlP poisoning are uprising as it is highly toxic and easily available for vulnerable groups as uneducated individual and teenagers.

Objective: This study aims to evaluate the ability of oxidative stress parameters including malondialdehyde (MDA), catalase activity and superoxide dismutase (SOD) to predict the outcome of AlP acute intoxication.

Patients and methods: This was a prospective cohort study on 50 cases of AlP-intoxicated cases who presented to Zagazig Poison Control Center, Emergency Department and Intensive Care Units of Zagazig University Hospitals from January 2021 to June 2021, forty-two of them were eligible to be included for age group (20-45). Serum samples were collected within 24 hours post ingestion.

Results: serum level of malondialdehyde (MDA) was significantly elevated and catalase activity was markedly declined in the non-survivors compared to the survivors, while SOD had no statistical value.

Conclusions: MDA and catalase activity showed good prognostic potential using statistical tests of comparison, although by receiver operating curves, they showed low specificity (41.2% and 76.5% respectively) with possibility of high false positive, while SOD was of no value.

Keywords: Oxidative stress parameters, Predict mortality in acute aluminum, Phosphide poisoning, Malondialdehyde.

INTRODUCTION

Aluminum phosphide "The wheat pill" is a fumigant which used to preserve grains widely in several developing agricultural countries, like Egypt, to protect wheat and rice from any infestation. The aluminum phosphide poisoning, whether accidental or suicidal, is considered a health hazard in Egypt nowadays; as it is a cheap and easy available poison without legal legislation controlling its purchasing [(1)].

The pill is composed of 56% aluminum phosphide and the rest are inactive ingredients. One pill contains about 1500 milligrams of aluminum phosphide[(2)].

The fatal dose for an average-sized individual is believed to be 150-500 milligrams**;** so less than half pill is enough to kill an adult individual as it is believed that aluminum phosphide is fatal when consumed from a recently opened container. Because of the immediate release of lethal amount of phosphine gas (PH3) when it comes in contact with the moisture or stomach content; then, phosphine gas is rapidly absorbed through lungs and stomach causing severe poisoning as it binds cytochrome oxidase and changes the valences of the hem component of hemoglobin (Hb)[(3, 4)].

The usual clinical picture of aluminum phosphide intoxication are nausea, abdominal pain, vomiting, hypotension, severe metabolic acidosis, cardiac arrhythmia, hepatic necrosis and congestive heart failure [(5)].

The main cause of death is the cardiogenic shock resistant to fluid therapy and inotropes followed by complications as disseminated intravascular coagulation (D.I.C) and multi organs failure [(2)].

With absence of specific antidote for aluminum phosphide poisoning the treatment of the AlP poisoning only is supportive and symptomatic [(6)].

This study aims to evaluate the ability of oxidative stress parameters including MDA, catalase activity and SOD to predict the outcome of AlP acute poisoning.

SUBJECTS AND METHODS

Prospective cohort study on fifty cases of AlP intoxicated cases who presented to Zagazig Poison Control Center, Emergency Department and Intensive Care Units of Zagazig University Hospitals from January 2021 to June 2021, forty-two of them were eligible to be included in represented inclusion age group (20-45).

Ethical consent:

The research was conducted with approval from a scientific research ethics commission Zagazig University (Institutional Research Board "IRB" number ZU-IRB #6665/13-1-2021).

Informed consent was taken from the patient's relatives or the patient himself when he was still conscious with keeping the patients` records confidential in all stages of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Inclusion criteria: History of certain intake of wheat pill within 24 hours. The cases presented with the

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clinical findings of acute aluminum phosphide poisoning, and the ages of the victims ranged between 20 to 45 years old.

Exclusion criteria: Cases with no clear history of exposure. Presence of co-morbidities or chronic diseases, and history of exposure to other drugs or toxin.

Sampling:

Ten centimeters of blood were collected from included patients on admission or within 24 hours from the intake and transferred to a plain tube and were left upright to clot then it was centrifuged for 10 minutes at 5000 rpm, the serum supernatant samples were collected and preserved at -80°C. samples were brought to room temperature again before performing of these tests.

Biochemical studies:

All cases` oxidative stress states were evaluated by measuring serum MDA, catalase activity and SOD by using commercial kits from Biodiagnostic, Egypt.

The absorbance of the resulting pink product was measured at 534 nm when malondialdehyde (MDA) interacts with thiobarbituric acid (TBA) in an acidic solution for 30 minutes [(7)].

While H_2O_2 was measured after reacting with catalase. The catalase inhibitor was added and the process was stopped after exactly one minute.

$$2\ H_2O_2 \xrightarrow{\text{catalase}} 2\ H_2O + O_2$$

The residual H_2O_2 used to interact to 4-aminophenazone (AAP) as well as 3,5-dichloro-2-hydroxybenzene sulfonic acid (DHBS) in the presence of peroxidase (HRP), producing a chromophore, whose colour intensity is inversely proportional to the quantity of catalase in the sample and was measured at 510 nm (500-520) [(8)].

The test for superoxide dismutase (SOD) hinges on SOD's capacity to prevent the reduction of nitro blue tetrazolium dye by phenazine methosulfate. It was discovered that purified SOD slowed down the initial pace at which O_2 was reduced to O_2 by photoactivated phenazine methosulfate-mediated reduction, hence slowing the reduction of nitroblue tetrazolium.

The purified enzyme caused 80% inhibition at 1.5 U/assay. The assessment was done by measuring the increase in absorbance at 560 nm for 5 minutes for control and sample at 25°C [(9)].

Study enrollment procedure:

Detailed history was taken from all cases including age, gender**.** Poisoning was confirmed by the history, which was taken from the victims and typical clinical presentation of toxicity**.** Assessment of vital signs and routine investigations were done to all cases. All cases received the management protocol of PCC-ZU.

Outcome measures:

All cases were followed up until full recovery or death. The outcome was categorized into survivors and non-survivors.

Statistical analysis

The data were analysed using SPSS, the Statistical Package for the Social Sciences, version 27.0. Frequencies and relative percentages were used to illustrate the qualitative data. Quantitative parametric data were expressed as mean±standard deviation (SD) and were compared by the independent T-test. While nonparametric quantitative data were expressed as median and interquartile range (IQR) and were compared by Mann Whitney (MW) test. Optimal cut-off values of various factors for maximal sensitivity and specificity in the prediction of an outcome were identified using receiver operating characteristic (ROC) curve analysis. P value < 0.05 was considered significant.

RESULTS

Forty-two patients were included in this study. Their age ranged between 20 and 45 years. Most cases were young females (Table 1).

Table (1): Demographic data of the studied cases

Variable		(n=42)	
Age: (year)	Mean ± SD	30±1.8	
Variable		**N**	**%**
SEX:	**Male**	9	21.5
	Female	33	78.5

SD: Standard deviation

Table (2) shows that there was a statistically significant impact of the MDA rise and catalase decline on the outcome, while there was no significant statistical impact between the superoxide dismutase level and the outcome (Figures 1, 2).

Table (2): statistical comparison of oxidative stress parameters (malondialdehyde, catalase and superoxide dismutase) between acute aluminium phosphide intoxication survivors and non-survivors

Variable		Survived (n=17)	Dead (n=25)	t/MW	P
MDA: (mmol/ml)	Mean ± SD	22.38±5.31	27.83±6.83	**2.41**	**0.02***
Catalase: (U/L)	Mean ± SD	307.6±71.35	186.9±41.31	**1.93**	**0.05***
SOD: (U/ml)	Mean ± SD	28.6±5.33	27.9±5.21	1.27	0.20

*: Significant

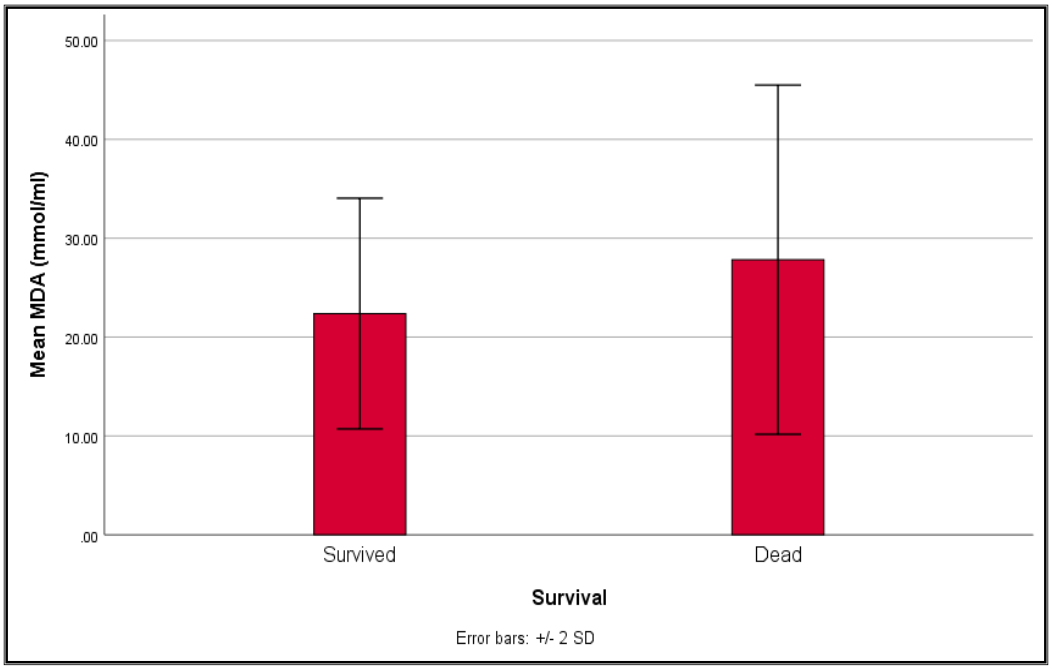

Fig. (1): Comparison of malondialdehyde (MDA) levels between acutely poisoned aluminum phosphide survivors and non-survivors

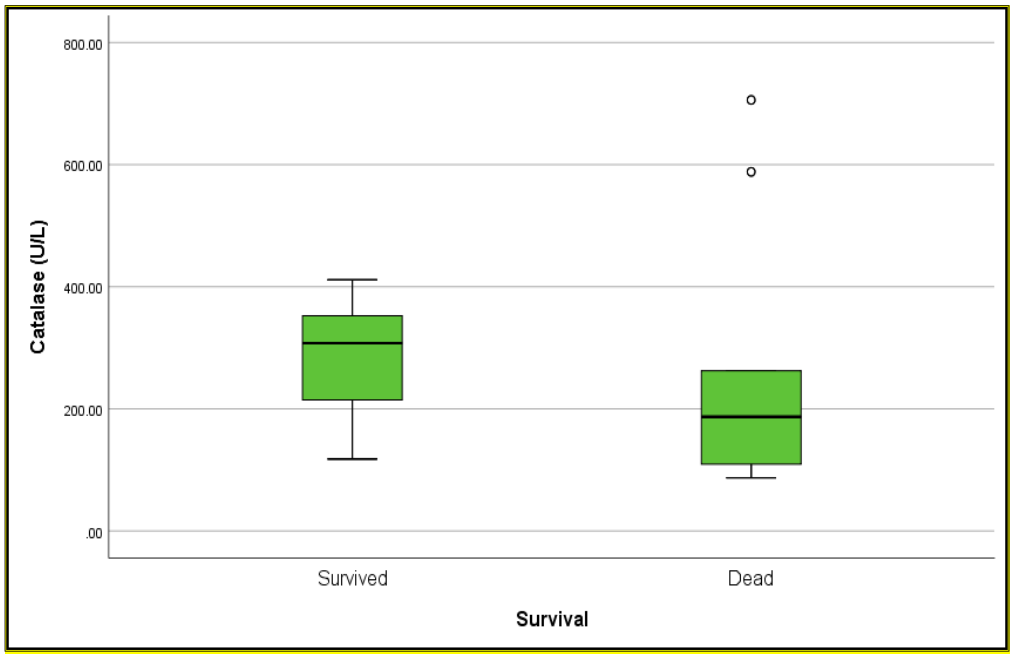

Fig. (2): Comparison of catalase levels between acutely poisoned aluminum phosphide survivors and non-survivors

The results of this study showed that all oxidative stress parameters according to the ROC curve were of poor accuracy in estimating mortality as shown in table 3 and figure 3.

Table (3): The validity of oxidative stress parameters (catalase, superoxide dismutase and malondialdehyde) in predicting mortality according to the ROC curve

	Cut off	AUC (95%CI)	P	Sens.	Speci.	PPV	NPV	Acc.
Catalase: (U/L)	<207.5	0.68 (0.51-0.85)	0.06	68	76.5	80.9	61.9	71.4
SOD: (U/ml)	<31.29	0.62 (0.45-0.79)	0.21	68	47.1	65.4	50	59.5
MDA: (nmol/ml)	<26.4	0.67 (0.50-0.84)	0.07	80	41.2	66.7	58.3	64.3

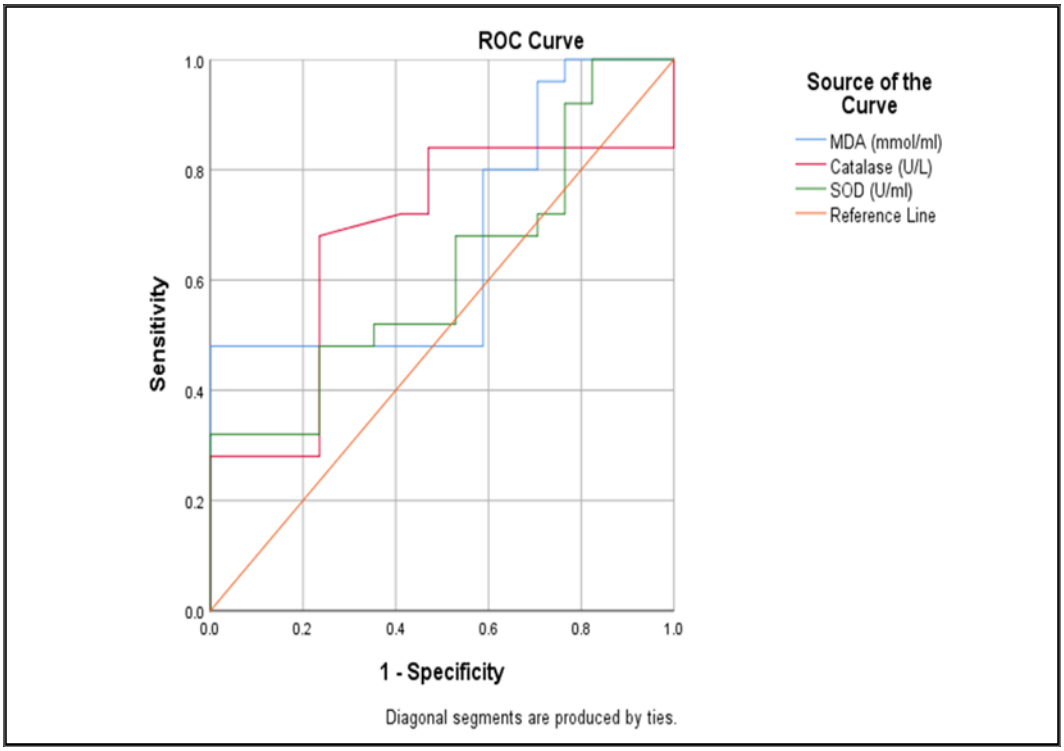

Fig. (3): Receiver operator characteristic curve (ROC) curve for Validity of oxidative stress parameters (Malondialdehyde, catalase and superoxide dismutase).

DISCUSSION

The pesticides are widely used in developing countries because of their ability to improve the quality and quantity of agricultural crops, but with absence of safety procedures and proper education the misuse of these chemicals became a health hazard. Aluminum phosphide is an important pesticide that is widely used in some countries including Egypt. Unfortunately, the AlP misuse as a suicidal tool has increased lately; as AlP is highly toxic and causes severe symptoms with a high mortality rate (40-80 %) [10, 11].

The wheat pill reacts with moisture and releases phosphine gas which induces oxidative damage by generation of highly reactive oxygen and hydroxyl species (ROS) beside inhibition of cytochrome c oxidase activity, catalase activity, decreasing level of reduced glutathione and decreasing the activity of superoxide dismutase (SOD) [10]. The increase of H_2O_2 production cause elevation in lipid peroxidation and significant rise in malondialdehyde (MDA); which is a marker of lipid peroxidation. These effects over the cellular enzymes remain for 5 days approximately [12].

Regarding the demographic data, the majority of the cases in this study were young females. This was similar to other studies performed in Egypt. This may be attributed to the low price of AlP and its easy availability without any legal restriction. In addition, young females are more psychologically vulnerable due to social, economic or family troubles [13, 14].

On the other hand, another study reported that males represented the majority of the acutely intoxicated AlP cases, and explained that due to occupational reasons; as only males can work in agriculture at their society[15].

In the present study, there was a relation between MDA and catalase activity and the outcome, as elevated MDA level and declined catalase activity are associated with poor outcome while SOD has no significant relation to the outcome. Another study declared that MDA significantly increases among the non-survivors [13]. The results showed no significant difference in SOD in both survivors and non survivors as well[16]. Moreover, there is an experimental study performed on rats and reported that the activity of catalase was lower among the rats that ingested a lethal dose of AlP with a significant increase in lipid peroxidation with elevation in the MDA production whereas superoxide dismutase was unaffected in the experimental rats[17].

However, other researchers found that catalase and SOD had a significant relation to the outcome and markedly decreased among the non survivors [18].

The detected rise of MDA and declination of catalase among the non survivors can be explained as aluminum phosphide (AlP) targeting the mitochondria and the main cause of toxicity is most probably due to mitochondrial dysfunction and interruption of the electron flow chain causing oxidative stress. Also, phosphine gas inhibits the catalase and peroxidase activity; thus, peroxide radicles accumulate in the body leading to rise in the hydroxyl radical-associated damage such as hydrogen peroxidation, which is associated with elevated MDA and a decline in the catalase [19].

The limitations of this study include the short duration of the study (only 6 months) with a relatively limited number of cases (42 cases).

CONCLUSION

This study observed the effect of the severe oxidative stress, which caused by acute AlP poisoning as it produces oxidative radicles causing consumption of the antioxidants besides, inhibition of the cellular protective mechanisms against oxidative stress. Serum level of malondialdehyde (MDA) was significantly elevated and catalase activity was markedly declined in the non-survivors compared to the survivors, although they were poorly specific when assessed by the ROC curves. While SOD was of insignificant value. Performing of larger studies is recommended to prove our results and to evaluate other parameters, which can be used to assess the prognosis of aluminium phosphide acute poisoning.

Conflict of interest: The authors declare no conflict of interest.

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Author contribution: Authors contributed equally in the study.

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