Research Article

Oxidative stress determined through the levels of antioxidant enzymes and the effect of N-acetylcysteine in aluminum phosphide poisoning

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Abstract

Introduction: The primary objective of this study was to determine the serum level of antioxidant enzymes and to correlate them with outcome in patients of aluminum phosphide (ALP) poisoning and, secondly, to evaluate the effect of N-acetylcysteine (NAC) given along with supportive treatment of ALP poisoning. **Design:** We conducted a cohort study in patients of ALP poisoning hospitalized at a tertiary care center of North India. The treatment group and control group were enrolled during the study period of 1 year from May 2011 to April 2012. **Interventions:** Oxidative stress was evaluated in each subject by estimating the serum levels of the enzymes, viz. catalase, superoxide dismutase (SOD) and glutathione reductase (GR). The treatment group comprised of patients who were given NAC in addition to supportive treatment (magnesium sulfate and vasopressors, if required), while in the control group, only supportive treatment was instituted. The primary endpoint of the study was the survival of the patients. **Measurements and Results:** The baseline catalase (P = 0.008) and SOD (P < 0.01) levels were higher among survivors than non-survivors. Of the total patients in the study, 31 (67.4%) expired and 15 (32.6%) survived. Among those who expired, the mean duration of survival was 2.92 ± 0.40 days in the test group and 1.82 ± 0.33 days in the control group (P = 0.043). **Conclusions:** This study suggests that the baseline level of catalase and SOD have reduced in ALP poisoning, but baseline GR level has not suppressed but is rather increasing with due time, and more so in the treatment group. NAC along with supportive treatment may have improved survival in ALP poisoning.

Keywords: Aluminum phosphide, catalase, N–acetylcysteine, glutathione reductase, superoxide dismutase

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Introduction

Each year, around 300,000 deaths occur worldwide due to pesticides.[1] Pesticide poisoning could be due to deliberate self-ingestion or accidental, occupational or for homicidal purpose, and is a global public health problem. Self-ingestion poisoning accounts for one-third of the world's suicide rate.[2] Aluminum phosphide (ALP) is a solid fumigant pesticide extensively used for household storage of grains. ALP poisoning has a high mortality rate that varies from 40% to 80%. However, the actual number could be much larger, as less than 5% cases of ALP poisoning eventually reach a tertiary care center.[3] It has now become one of the most common causes of poisoning among agricultural pesticides.[4] ALP is also a potential threat for chemical terrorism due to its immediate effect of releasing lethal phosphine gas.[5] Phosphine inhibits mitochondrial cytochrome oxidase and inhibits oxidative respiration.[6] In ALP poisoning, superoxide and peroxide radicals are formed that lead to cellular membrane damage by lipid peroxidation.[7] Post-mortem, the lower levels of superoxide dismutase (SOD), malonyldialdehyde and catalase are found and are related to mortality. These

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biochemical parameters have attained a normal profile in survivors by Day 5 and connote a restriction of oxidative stress following phosphine elimination.[8]

A study conducted by Azad *et al*. has demonstrated the beneficial effect of N-acetylcysteine (NAC) in ALP poisoning in rats by reducing myocardial free oxygen radical injury and increasing the survival time in rats.[9]

The role of NAC in ALP poisoning has not been clear. In this study, we evaluated the serum level of antioxidant enzymes and correlated them with outcome in patients of ALP poisoning and, secondly, evaluated the effect of NAC given along with supportive treatment of ALP poisoning in parallel with supportive treatment.

Materials and Methods

Design and setting

We conducted a cohort study (NAC-treated group vs. non-treated group) in patients of ALP poisoning at a tertiary care center of North India. The treatment group and control group were enrolled during the study period of 1 year from May 2011 to April 2012. Subjects were confirmed victims of ALP poisoning admitted to our center, and the confirmation was performed by documentation of materials and by using the silver nitrate test.

Interventions

The oxidative stress was evaluated in each subject by estimating the serum levels of the enzymes, viz. catalase, superoxide dismutase (SOD) and glutathione reductase (GR). Patients in the treatment group received NAC at a dose of 150 mg/kg iv over I h in 200 mL 5% dextrose, followed by 50 mg/kg in 500 mL over 4 h, followed by 100 mg/kg in 500 mL over 16 h [5] in addition to other supportive treatment (magnesium sulfate, 70 mmol/L over 24 h;[5] vasopressors if required) and in the control groups only supportive treatment was given (magnesium sulfate, 70 mmol/L over 24 h; vasopressors if required). A third group of healthy subjects was taken to estimate the reference range for oxidative markers but not for comparison of the other parameters. Excluded from the study were those who were using NAC for other diseases, especially chronic obstructive pulmonary disease, cases of multiple concomitant poisoning, patients who had undergone cardiopulmonary resuscitation, chronic kidney disease, chronic liver diseases, chronic heart failure and those who refused to give consent. A detailed history and clinical examination was performed in each subject. Arterial blood gas analysis with serum levels of lactate and levels of antioxidant enzymes, which consisted of catalase, SOD and GR, were estimated in addition to routine investigations, which included complete blood picture, blood sugar, electrolytes, urea, creatinine and liver function tests in each patient. The antioxidant enzymes were estimated by a technique common for all, which was to prepare the lysate by centrifuging the blood sample at 3000 revolution per minute (rpm) for 15 min and discarding the supernatant plasma and pellet (consisting of RBCs). Wash twice with 1 mL normal saline (0.9% NaCl) and centrifuge at 3000 rpm for 10 min, discard the supernatant (normal saline) then centrifuge the pellet + chilled distilled water (equal volume to plasma) at 5000 rpm for 20 min. The catalase levels were estimated after a common procedure by the method described by Aebi, 1984.[10] SOD and GR were estimated by the method described by Mc Cord and Fridovich, 1969[11] and Hazelton and Lang, 1985.[12] The primary end point was the survival of the patient. The study was approved by the ethical committee of our institute.

Data collection and variables

The data were presented in numbers, frequency and mean. Data were analyzed statistically by SPSS 17 and paired *t* test and the Chi-square test were used. A $P < 0.05$ for a 2-sided test was considered statistically significant. Data were regressed to analyze the variable associated with the outcome. An ROC curve was drawn and the criteria for acceptability for area under cover (AUC) was considered as defined by Hosmer and Lomeshaw.

Results

The present study encompassed a total of 46 subjects of ALP, of whom 24 were in the treatment group and 22 were controls. The mean age of the patients was 27.74 ± 8.86 years (range 15–60 years). Males were 17 (70.8%) in the treatment group and 16 (72.7%) in the control group. Subjects directly reaching our center without taking any medical treatment in between were 13 (54.2%) in the test group and 15 (68.2%) in the control group ($P = 0.331$). At admission, 20 patients in each group, comprising 87% of the total patients, presented with hypotension and received vasopressors. Baseline demographic and clinical characteristic were similar in both groups [Table 1]. Except for the mean potassium levels, which were significantly higher in the control group as compared with the treatment group, other laboratory characteristics were similar in both groups [Table 2]. Similarly, the mean levels of oxidative enzymes were not different between the two groups [Table 3].

Table 1: Demographic and clinical characteristics in the treatment and control groups

Characteristics	Treatment group (*n*=24)	Control group (*n*=22)	*P* value
Age group (in years)			
15-25	13 (54.2)	9 (40.9)	
26-35	10 (41.7)	10 (45.5)	
>35	1 (4.2)	3 (13.6)	0.440
Gender			
Male	17 (70.8)	16 (72.7)	
Female	7 (29.2)	6 (27.3)	0.887
Referred cases	11 (45.8)	7 (31.8)	0.331
Direct case	13 (54.2)	15 (68.2)	0.331
Primary treatment received	12 (50.0)	6 (27.3)	0.115
Hypotensive patient	20 (83.3)	20 (90.9)	0.445
Altered sensorium	1 (4.2)	2 (9.1)	0.439

Table 2: Baseline clinical and laboratory parameters between the treatment and control groups were compared at the time of admission

Parameters	Treatment group (*n*=24)		Control group (*n*=22)		*P* value
	Mean	SD	Mean	SD	
Hematological parameters					
Hemoglobin (gm %)	12.9	3.5	12.8	2.8	0.956
Total leukocyte counts (cells/mm^3)	7.92	3.38	7.56	3.22	0.895
Platelet counts (lacs/mm^3)	1.6	0.5	1.5	0.5	0.499
Biochemical parameters					
RBS (mg/dL)	127.5	99.2	135.1	104.8	0.660
Na^+ (mmol/L)	139.7	8.5	138.5	8.0	0.537
K^+ (mmol/L)	3.6	0.7	4.4	1.5	0.049
Urea (mg/dL)	35.9	15.6	43.5	36.2	0.965
Creatinine (mg/dL)	0.9	0.3	1.2	1.1	0.850
Serum bilirubin (mg/dL)	1.5	1.0	1.9	1.5	0.338
SGOT (u/L)	101.2	148.8	92.2	74.9	0.855
SGPT (u/L)	92.9	85.0	106.1	125.5	0.618
SALP (u/L)	174.3	58.8	169.5	71.1	0.276
Serum protein (mg/dL)	5.9	0.8	5.6	1.0	0.402
Serum albumin (mg/dL)	3.4	0.7	3.1	0.7	0.202
ABG analysis					
PH	7.2	0.2	7.2	0.2	0.930
PCO_2 (mmHg)	31.3	15.1	33.7	12.0	0.327
PO_2 (mmHg)	70.2	34.2	63.2	31.6	0.265
HCO_3 (mmol/L)	13.7	6.1	14.1	7.8	0.886
Lactate (mmol/L)	9.8	4.8	10.8	4.1	0.708
Oxidative stress markers					
Catalase (unit/min/mg protein)	3.8	6.0	5.7	8.8	0.767
Superoxide dismutase (unit/mL)	18.3	9.9	17.7	10.4	0.809
Glutathione reductase (unit/min/mg protein)	36.2	29.2	36.1	24.5	0.912

SD: Standard deviation; RBS: random blood sugar; SGOT: Serum glutamic oxaloacetic transaminase; SGPT: Serum glutamic pyruvic transaminase; SALP: serum alkaline phosphatase

Around 2/3rd of the total patients died (31/46). In the test group, patients surviving were 45.8% (11/24), while 18.2% (4/22) survived in the controls ($P = 0.046$). As shown in Figure 1, the mean duration of survival among those who died was 2.92 ± 0.40 days in the test group and 1.82 ± 0.33 days in the control group ($P = 0.043$).

Table 3: Comparison of the mean levels of different antioxidant enzymes between the two groups

Oxidative markers (reference value)	Time intervals (days)	Control (*n*=22)		Treatment (*n*=24)		*P* value
		Mean	SD	Mean	SD	
Catalase (16.5±9.5 x 10^{-3} unit/min/mg protein)	0	3.9	6.2	3.8	6.0	0.367
	1	9.7	9.2	8.4	8.6	0.235
	5	13.6	10.5	12.1	10.7	0.226
Superoxide dismutase (31±8.52 unit/mL)	0	17.7	10.4	18.3	9.9	0.809
	1	21.2	12.9	29.6	9.4	0.362
	5	26.3	5.8	37.7	5.7	0.435
Glutathione reductase (24.2±7.4 x 10 unit/min/mg protein)	0	36.1	24.5	36.2	29.2	0.912
	1	33.5	35.3	57.4	30.5	0.102
	5	51.8	41.1	71.9	32.4	0.374

SD: Standard deviation

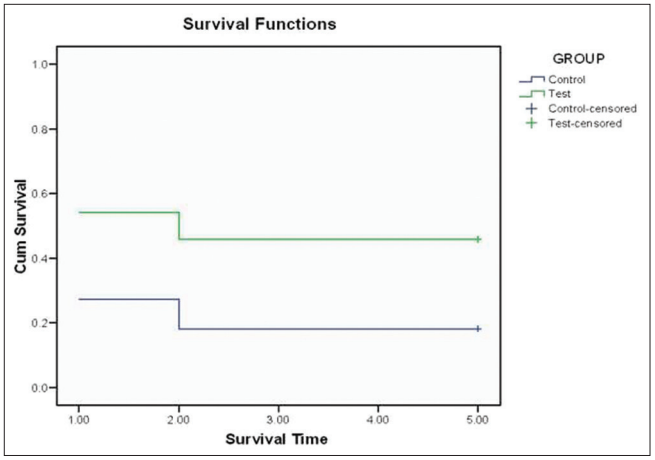

Figure 1: Survival function plot shows survival time is higher in the test group than the control group among patients who died

In the subgroup analysis, HCO3– levels were higher in survivors at admission (Day 0), while pO_2 was higher at Day 1 and Day 5 in the survivors and lactate concentration was lower in survivors at Day 0 and at Day 1 [Table 4].

Baseline catalase and SOD levels were significantly higher among survivors as compared with those who expired, but GR levels did not differ between them [Table 5]. At Day 1, GR levels were higher in those who survived ($P = 0.017$). Antioxidant enzymes could not be measured among those who expired at Day 5.

The area under curve (AUC) for lactate levels at Day 1 ($P < 0.001$) with respect to mortality was 0.860, which was much above the described acceptability criteria of 0.7. A cut-off value >6.90 mmol/L was regressed to be 100% sensitive and 73.3% specific for the outcome [Figure 2]. AUC for catalase ($P = 0.008$) and SOD ($P = 0.001$) was well above the criteria for acceptability as defined by Hosmer and Lomeshaw. A cut-off value of catalase < 1.92 unit/min/mg protein/day was regressed to be 74.2% sensitive and 73.3% specific

Table 4: Comparison of ABG parameters between two outcome groups at different time intervals

ABG parameters	Time intervals (in days)	Survived ($n=15$)		Expired ($n=31$)		*P* value
		Mean	SD	Mean	SD	
pH	0	7.3	0.1	7.2	0.2	0.066
	1	7.4	0.1	7.3	0.2	0.432
	5	7.4	0.0	7.2	0.3	0.067
	0	35.2	9.0	31.1	15.4	0.069
	1	38.2	5.6	31.4	7.6	0.087
pCO_2	5	45.0	6.9	35.5	4.9	0.067
	0	17.6	4.6	12.1	7.2	0.008
-	1	20.9	3.2	15.4	9.4	
HCO_3	5	27.1	6.5	15.6	11.4	0.391
	0	87.1	31.2	75.7	38.3	0.067
	1	81.4	14.0	53.2	17.3	0.271
pO_2	5	92.0	7.4	47.5	9.2	0.003
	0	5.8	4.6	12.4	2.3	0.017
Lactate	1	2.8	1.6	12.7	1.5	<0.001
	5	1.48	0.57	-	-	0.001

ABG: Arterial blood gas; SD: Standard deviation

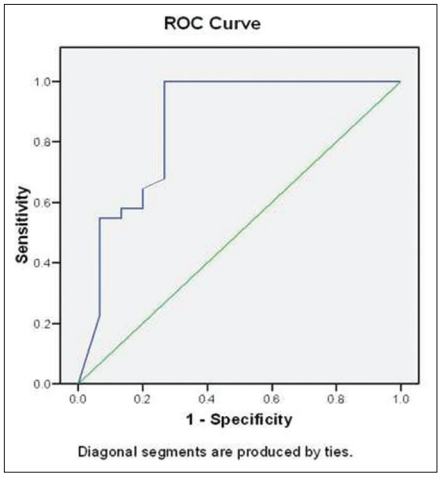

Figure 2: The receiver operating characteristic curve for lactate area under curve at Day 1 ($P < 0.001$) with respect to mortality are 0.860, well above the criteria of acceptability value of 0.7. A cut-off value of lactate >6.90 mmol/L is 100% sensitive and 73.3% specific for mortality

for prediction of expiry, whereas for SOD a cut-off value of <18.9 units/mL was regressed to be 90.3% sensitive and 86.7% specific for prediction of mortality. As AUC for GR was below 0.7, which is below the acceptability criteria, it was not further evaluated [Figure 3]. The blood lactate level showed the maximum sensitivity as well as accuracy of prediction (100% sensitivity and 91.3% accuracy), followed by SOD with excellent sensitivity (90.7%) as well as specificity (86.7%) and catalase with limited sensitivity (74.2%) as well as specificity (73.3%).

Discussion

The present study shows that NAC (treatment group) along with supportive treatment has improved the

Table 5: Comparison of oxidative stress markers between two outcome groups at different time intervals

Antioxidant enzymes	Time intervals (in days)	Survived ($n=15$)			Expired ($n=31$)			*P* value
		No.	Mean	SD	No.	Mean	SD	
Catalase	0	15	10.4	10.3	31	2.0	3.1	0.008
	1	15	12.3	11.2	3	1.9	3.1	0.056
	5	15	15.2	12.7				
	0	15	28.4	7.1	31	13.0.	6.9.	<0.001
	1	15	32.1	6.1	3	14.2	15.7	0.076
Superoxide	5	15	37.7	5.5				
dismutase	0	15	46.7	29.9	31	31.0	23.9	0.064
	1	15	56.8	31.5	3	12.8	2.4	0.017
Glutathione	5	14	66.2	34.7				
reductase								

SD: Standard deviation

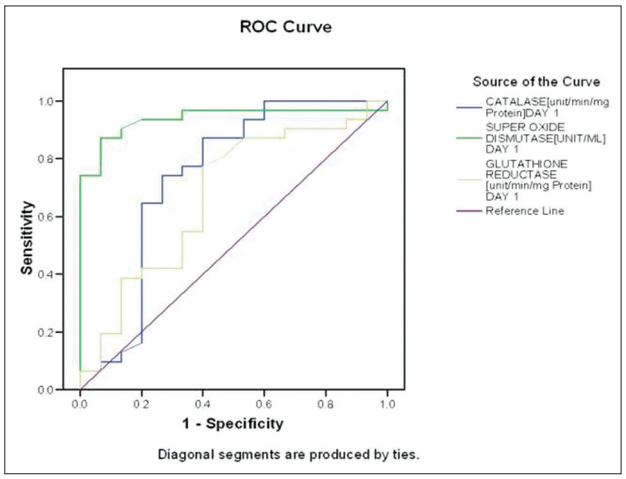

Figure 3: The receiver operating characteristic curve for enzymes, area under curve for catalase ($P = 0.008$) and superoxide dismutase ($P = 0.001$) are well above the criteria for acceptability as defined by Hosmer and Lomeshaw. However, area under curve for glutathione reductase is below 0.7, which is below the acceptability criteria

primary outcome over supportive treatment given alone (control group) in patients of ALP poisoning. The mean duration of survival is increased in the treatment group as compared with the control group. Azad *et al.*, in a study on rats, have reported that treatment with NAC in ALP poisoning significantly increased the survival time, early stabilization of blood pressure, heart rate and ECG by reducing myocardial oxidative injury.[9]

The second objective of the study was to evaluate the level of antioxidant enzymes (catalase, SOD and GR) in both groups. In both groups, catalase and SOD activity were reduced at presentation, while the activities of GR were within the normal range. However, SOD and GR levels were approximately increased to twice the initial level to the level measured on Day 5 in the treatment group. It also indirectly favors fact that NAC increases the GR activity in the treatment group, while the level of catalase did not change significantly in both the groups.

Chugh *et al.* noticed that the level of catalase decreased in patients of ALP poisoning.[13]

The levels of antioxidant enzymes (catalase and SOD) were significantly low in patients who died. But, the GR level in the expired group was not reduced on the day of presentation. On Day 1, GR activity was decreased, which indirectly suggests that ALP toxicity affects GR activity maximally by Day 1. Chugh *et al.* have shown that indicators of oxidative stress reach peak levels within 48 h of exposure of poison, approaching normalization by Day 5.[8]

AUC for catalase and SOD were well above the criteria for acceptability as defined by Hosmer and Lomeshaw and were associated with mortality ($P < 0.05$). A cut-off value of catalase ≤1.92 unit/min/mg protein/day was regressed to be 74.2% sensitive and 73.3% specific for prediction of expiry, whereas for SOD a cut-off value ≤18.9 was regressed to be 90.3% sensitive and 86.7% specific for prediction of expiry. The AUC for Day 1 lactate levels with respect to mortality was 0.860, which is much above the described acceptability criteria of 0.7. A cut-off value ≥ 6.90 mmol/L was regressed to be 100% sensitive and 73.3% specific for the outcome. Among all these three enzymes, SOD had maximum area under ROC curve ($P < 0.001$; CI 0.866–1.011) for prediction of mortality. Moreover, lactate was also an important parameter that could predict mortality–the ROC curve area was 0.86 ($P < 0.001$; CI 0.724–0.996).

Demographic and clinical characteristics of the patients were similar to reduce bias in the study. The present study shows that most of the patients in our study were young (under 35 years of age) and predominantly male, it may be due to easy accessibility of males to ALP compounds because they work in the agriculture fields and storage house of grains where it is most commonly used. It was also observed in a study conducted by Mathai *et al.*[2] that majority of the patients were young males. As reported by Gunnell *et al.*,[1] self-poisoning is the most common method of poisoning; in their study, most of the cases were suicidal and few cases were accidently exposed to ALP. As the study was conducted at a tertiary care center, the number of referred cases should ideally be more as compared with direct cases; however, the number of direct cases was higher as compared with the referred cases. The could be due to the high fatality rate of this poisoning, which lead to early demise of the patient before they could reach the tertiary care center. A study has already shown that only less than 5% patients of those with ALP poisoning eventually reach a tertiary care center.[3]

In the present study, shock was the most common clinical finding, and the shock was refractory to inotropes in most of the patients. ALP poisoning causes suppression of the cardiac system and functions of vascular tissues, resulting in profound and refractory hypotension. Shock has been also described as the most common manifestation and is a leading cause of death. Some patients also presented in altered sensorium. These patients were also in a state of shock, which might have been the reason for the altered sensorium. On analysis, ABG parameters and baseline hematological and biochemical parameters between the test and control groups were similar. In both group, patients were hypoxic and had metabolic acidosis due to the increased level of lactate. Dua *et al.* have also observed an increased level of lactate in rats with ALP.[14]

Both group showed severe metabolic acidosis at the time of presentation, which may be due to the accumulation of lactic acid caused by blockage of oxidative phosphorylation and poor tissue perfusion. A study by Jaiswal *et al.* has shown that all the patients admitted with ALP poisoning were in severe metabolic acidosis.[15]

Moreover, ABG analysis showed that expired patients had severe metabolic acidosis, tachypnea, higher lactate levels and more hypoxia as compared with those who survived. The lactate level (>6.9 mmol/L) had 100% sensitivity in predicting the mortality at the time of presentation. Only HCO_3 and lactate levels at the time of presentation showed a significant association with outcome.

The limitations of the study were small sample size in both groups and free radicals level that was not determined along with the antioxidant enzymes. Yet, we would like to conclude that NAC along with supportive treatment may have a survival benefit over supportive treatment alone for outcome in such highly fatal ALP poisoning. The initial level of catalase and SOD was reduced in both groups. The GR activity had initially not decreased; instead, its activity increased with due time in both groups, but more in the test group. In patients who expired, the levels fell markedly on Day 1. Overall, ALP poisoning has high mortality. Lactate levels >6.9 mmol/L at the time of admission shows 100% sensitivity in prediction of mortality, followed by SOD and catalase.

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