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Research Article

Accuracy of Using Glutamate Dehydrogenase (GDH) and High-Mobility Group Box-1 (HMGB1) Plasma Levels as Early Predictors of Acute Aluminum Phosphide Hepatotoxicity

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Abstract

Aluminum phosphide (Alp) poisoning is one of commonest causes of hepatotoxicity in Minia Governorate. Glutamate dehydrogenase (GDH) and high-mobility group box-1 (HMGB1) are liver biomarkers that reflect structural liver damage.

Subjects and methods: The current study was conducted at Minia Poisoning Control Centre during the period from January, 1st, 2015 till December, 31st, 2015, where 28 patients presented with acute oral AlP poisoning. All subjects were investigated at 12, 24, 36 & 48 hours post ingestion for serum GDH and liver function tests (ALT, AST and ALP).

Results: The results revealed that serum GDH significantly increased in the first 12 hours samples, while the other liver function tests and HMGB1 were not significantly affected. In addition, it was reported that serum GDH, HMGB1 and liver function tests (ALT, AST, and ALP) were significantly increased after 24 and 36 hours post-ingestion. All measured parameters started to decline in the 48 samples, but still significantly increased.

Conclusion: Basing on the result of this study it could be concluded that GDH is a novel hepatic biomarker that could be used as an early predictor of AlP hepatotoxicity rather than the other investigated parameter. It is suggested to conduct a bigger study that involves multi-centers to assure this specificity and accuracy.

INTRODUCTION

Aluminum phosphide (AlP) is a solid fumigant which has been extensively used since the 1940s. It is easily available [1]. In Egypt, it is known as the wheat tablet that can be purchased in local shops.

AlP is a solid pesticide that rapidly became one of the most commonly used grain fumigants because of its properties which are considered to be near ideal; it is toxic to all stages of insects, highly potent, does not affect seed viability, is free from toxic residues and leaves little residue on food grains [2].

This highly toxic chemical emerges as a poison of suicidal deaths as this pesticide has no effective antidote and is freely available in the market [3]. Hepatotoxic effects of AlP have been reported in the form of jaundice and altered transient elevation of serum aspartate and alanine aminotransferase [4-6].These markers, though useful for tracking the overall progression and resolution of organ damage, provide little or no insight into mechanisms of hepatotoxicity. They are also limited in prognostic utility. Since by definition they follow, rather than precede, the tissue damage, they cannot be used to predict patient outcome at early time points [7]. Hence, the need to find new biomarkers that can be utilized in such cases to diagnose and predict the patient outcome. The current study was conducted to evaluate the effects of AlP on serum HMGB1 and GDH and the accuracy of using these biomarkers to predict the acute hepatotoxic effects of AlP.

SUBJECTS AND METHODS

The current study was conducted at Minia Poisoning Control Centre during the period from January, 1st, 2015 till December, 31st, 2015, where 28 patients who presented with acute oral AlP poisoning. Another 20 apparently healthy volunteers were subjected to same investigations as a control group.

All subjects had no history of concomitant administration of another drug(s), systemic diseases, bilharziasis, hepatitis, nor malignancies.

All subjects received the usual treatment including methods of elimination (Emesis, activated charcoal, etc). All subjects were

investigated at 12, 24, 36 & 48 hours post ingestion for serum glutamate dehydrogenase, HMGB1 and liver function tests (ALT, AST and ALP).

Biochemical investigations

Liver function tests: Alanine transferase and Aspartate transferase (ALT and AST): were measured spectrophotometrically using Spekol II Carl-Zeiss spectrophotometer [8,9]. Serum alkaline phosphatase level (Alk. P.), measured by coloremetric method according to Donald & Ralph, 1993 [10].

Glutamate dehydrogenase: It is measured using a Hitachi 917 automated clinical chemistry analyzer (Roche Diagnostics Limited, Lewes, UK).

Plasma level of HMGB1: It was determined by enzyme-linked immunosorbent assays according to the manufacturers' instructions (Human ELISA kit, Uscn Life Science Inc, Wuhan, China). All samples were run in duplicate and repeated if there was a >15% difference between duplicates. Intra-assay coefficient of variance (CV) was 10% and inter-assay CV of 12%. No significant cross-reactivity or interference was observed.

Statistical analysis

The collected data was organized, tabulated and statistically analyzed using SPSS software statistical computer package version 10. Data were expressed as Mean + Standard Deviation (SD). Student-*t* test was used to differentiate between two means where probability (P); $P < 0.05$: was considered significant. Correlations were estimated using Pearson's test where probability (P): $P < 0.05$ was considered significant.

RESULTS

From the 28 adult patients with a history of AlP toxicity only 19 patients survived. All deaths occurred 12-18 hours post-ingestion. The results of the current study revealed that serum GDH significantly increased in the first 12 hours samples, while the other liver function tests and HMGB1 were not significantly affected. In addition, it has been found that the level of serum GDH, HMGB1 and liver function tests (ALT, AST, and ALP) were significantly increased after 24 and 36 hours post-ingestion. All measured parameters started to decline in the 48 hours samples, but still significantly increased for all of them (Tables 1-5 & Figures 1-3).

Comparing the results of the non-survivors to those of the survivors at 12 hours post-ingestion revealed a very highly significant difference regarding the all measured parameters (Table 6 and 7).

DISCUSSION

AlP is a highly toxic cheap rodenticide which, on exposure to moisture, liberates phosphine gas. Phosphine gas is well-known to be rapidly absorbed by inhalation, dermally, or gastrointestinally [11].

Reviewing the archive of Minia PCC, it has been reported that AlP poisoning is one of commonest causes of poison-induced hepatotoxicity in Minia Governorate. Due to the lack of specific antidote of such poison and the fact that management of such cases is still symptomatic and supportive and that the survival of the patients is unlikely if more than 1.5 gm is ingested [12], the need for rapid diagnosis and intervention is of great importance.

Hepatotoxic effects of AlP have been reported in the form of jaundice and altered transient elevation of serum aspartate and alanine aminotransferase [4-6]. These markers, though useful for tracking the overall progression and resolution of organ damage, provide little or no insight into mechanisms of hepatotoxicity.

The current study was conducted to evaluate the effects of AlP on serum HMGB1 and GDH and the accuracy of using these biomarkers to predict the acute hepatotoxic effects of AlP.

The results of this study revealed that serum GDH significantly increased in the first 12 hours samples, while the other liver function tests and HMGB1 were not significantly affected. In addition, it has been found that serum GDH, HMGB1 and liver function tests (ALT, AST, and ALP) were significantly increased after 24 and 36 hours post-ingestion. All measured parameters started to decline in the 48 samples, but still significantly increased for all of them.

The findings regarding aminotransferases are concomitant with the previously reported ones that stated that, in the rat, these enzymes have theoretical disadvantages as hepatic biomarkers since they have a relatively low intrahepatic activity and short half-life in blood compared with that in the dog, monkey, and man [13]. Furthermore, a recent study indicates that in the rat, plasma ALT may fail to indicate hepatic necrosis [14].

Regarding HMGB1, the results were not surprising as it exerts its effects through a number of Toll-like receptors (TLR2/4), and this leads to the activation of immune cells and

Table 1: Values of ALT, AST, ALP, Bilirubin, GDH and HGMB1 of acute aluminum phosphide intoxicated patients (Mean + S.D).

Time (Hrs)	Parameter				
	ALT (mg/dl)	AST (mg/dl)	ALP (mg/dl)	GDH (U/l)	HGMB1 (ng/mL)
	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D	Mean ± S.D
Volunteers (n=20)	28.58±5.81	30.92±4.21	21.12±3.97	22.86±6.91	11.98±3.83
Non-survivors 12 hrs (No.=9)	56.34± 6.51	59.76±7.18	71.44±27	571.24±35.63	20.53±4.28
Survivors					
12 hr (No.=19)	29.81 ± 6.41	31.52 ± 4.52	21.38± 4.14	212.13±11.3	12.53 ± 3.23
24 hr (No.=19)	178.39 ± 14.72	186.28 ± 13.39	71.36 ± 6.62	316.87±15.7	18.25 ± 5.72
36 hr (No.=19)	236.22 ± 28.37	241.27 ± 16.38	96.27 ± 8.24	532.76±21.4	21.11 ± 7.84
48 hr (No.=19)	118.45 ± 13.26	119.11 ± 11.52	69.51 ± 7.26	345.23±12.8	16.37 ± 4.56

Table 2: Comparison between the values of ALT (mg/dl) in the different estimated post-ingestion intervals.

Time (Hrs)	Control		12		24		36		48	
	t-value	P-value	*t*-value	P-value	*t*-value	P-value	*t*-value	P-value	*t*-value	P-value
12	0.63	0.534	-	-						
24	42.2	<0.001	40.3	<0.001	-	-				
36	32.1	<0.001	30.9	<0.001	7.89	<0.001	-	-		
48	27.7	<0.001	26.2	<0.001	13.2	<0.001	16.4	<0.001	-	-

Student-*t* test; P < 0.05 significant

Table 3: Comparison between the values of AST (mg/dl) in the different estimated post-ingestion intervals.

Time (Hrs)	Control		12		24		36		48	
	t-value	P-value	*t*-value	P-value	*t*-value	P-value	*t*-value	P-value	*t*-value	P-value
12	0.43	0.670	-	-						
24	49.4	<0.001	47.7	<0.001	-	-				
36	55.6	<0.001	53.8	<0.001	11.3	<0.001	-	-		
48	32.1	<0.001	30.9	<0.001	16.6	<0.001	26.6	<0.001	-	-

Table 4: Comparison between the values of ALP (mg/dl) in the different estimated post-ingestion intervals.

Time (Hrs)	Control		12		24		36		48	
	t-value	P-value	*t*-value	P-value	*t*-value	P-value	*t*-value	P-value	*t*-value	P-value
12	0.2	0.842	-	-						
24	28.9	<0.001	27.9	<0.001	-	-				
36	36.6	<0.001	35.4	<0.001	10.3	<0.001	-	-		
48	26	<0.001	25.1	<0.001	0.82	0.917	10.6	<0.001	-	-

Student-*t* test; P < 0.05 significant

Table 5: Comparison between the values of GDH (U/l) in the different estimated post-ingestion intervals.

Time (Hrs)	Control		12		24		36		48	
	t-value	P-value	*t*-value	P-value	*t*-value	P-value	*t*-value	P-value	*t*-value	P-value
12	63.5	<0.001	-	-						
24	76.4	<0.001	23.6	<0.001	-	-				
36	101	<0.001	57.8	<0.001	35.5	<0.001	-	-		
48	98.6	<0.001	34	<0.001	6.1	<0.001	32.8	<0.001	-	-

Student-*t* test; P < 0.05 significant

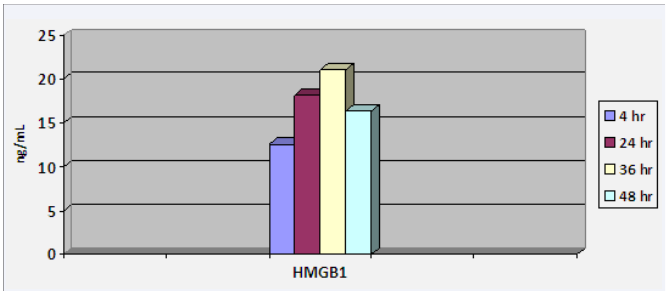

Figure 1 Values of ALT, AST, ALP of acute aluminum phosphide intoxicated patients (Mean + S.D).

the consequent release of multiple pro-inflammatory cytokines [15]. In animal models of infection or local tissue injury, HMGB1 functions as a critical mediator of systemic or local inflammatory injury [16]. These processes are time-consuming and as a result, HMGB1 has been established as a late mediator of lethal systemic inflammation. Recent studies have demonstrated that hepatocytes can actively release HMGB1 after being challenged with some poisons and HMGB1 cytoplasmic translocation was observed in liver cells in an animal model of acute liver failure (ALF) induced by D-galactosamine (D-GalN), as well as in patients with ALF [17].

On the other hand, the reported early increase of serum GDH the first 12 is in accordance with O'brien and his fellows, 2002, who reported that GDH has been increased several-fold in most rats treated with either dexamethasone or cyproterone despite having no histopathological evidence of hepatocellular necrosis [18]. This could be explained by the fact that GDH is synthesized in the cytoplasm [19], so, there will be at least residual GDH

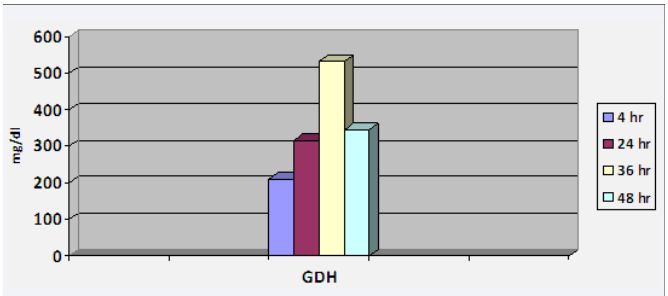

Figure 2 Values of serum GDH activity of acute aluminum phosphide intoxicated patients (Mean + S.D).

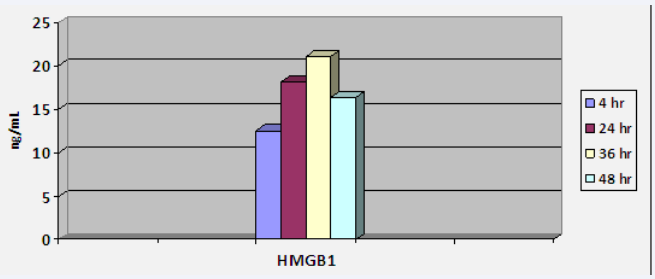

Figure 3 Values of serum HGMB1 activity of acute aluminum phosphide intoxicated patients (Mean + S.D).

Table 6: Comparison between the values of HGMB1 (ng/mL) in the different estimated post-ingestion intervals.

Time (Hrs)	Control		12		24		36		48	
	t-value	P-value	*t*-value	P-value	*t*-value	P-value	*t*-value	P-value	*t*-value	P-value
12	0.48	0.632	-	-						
24	4.04	<0.001	3.8	0.001	-	-				
36	4.38	<0.001	5.79	<0.001	1.28	0.207	-	-		
48	3.26	0.002	3	0.005	1.12	0.270	2.28	0.029	-	-

Student-*t* test; P < 0.05 significant

Table 7: Correlation between the different measured parameters among the survivor and non-survivor intoxicated patients.

	Non-survivors (n=9)	Survivors (n=19)	Control (n=20)	P1	P2	P3
ALT (mg/dl)	56.3± 6.51	29.81 ± 6.41	28.58±5.81	<0.001	<0.001	0.538
AST (mg/dl)	59.7±7.18	31.52 ± 4.52	30.92±4.21	<0.001	<0.001	0.709
ALP (mg/dl)	71.4±27	21.38± 4.14	21.12±3.97	<0.001	<0.001	0.946
GDH (U/l)	571.2±35.63	212.13±11.3	22.86±6.91	<0.001	<0.001	<0.001
HGMB1(ng/mL)	20.5±4.28	12.53 ± 3.23	11.98±3.83	<0.001	<0.001	0.644

P1: Non Survivor vs survivor; P2: Non Survivor vs Control; P3: Survivor vs Control
Pearson's test; P < 0.05 significant

available for release into plasma.

In addition, blebbing, the primary mechanism by which the hepatocellular release of enzymes from prelethally-injured cells into plasma occurs, is thought only to release cytosolic content. Furthermore, blebs may contain small amounts of mitochondrial material, which may be released into the plasma [20].

Basing on the result of the present study, it could be concluded that GDH is a more effective novel hepatic biomarker that could be used in early prediction of acute ALP-induced hepatic injury rather than ALT, AST, SDH or ALP in the rat, based primarily on the large increase following hepatocellular injury, prolonged persistence in the blood following injury, high sensitivity for detection of injury (including pre-necrotic injury), high tissue specificity, and lower susceptibility to inhibition or induction. It is suggested to conduct a bigger study that involves multi-centers to assure this specificity and accuracy.

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