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Assessment of the Role of Keratin 18 and High Mobility Group Box 1 Biomarkers for Early Prediction of Drugs and Chemicals Induced Hepatotoxicity: A Prospective Study in the Poison Control Center of Ain Shams University Hospitals (PCC-ASUH)

Ghada Mohamed Ebeid[1], Manal El Sayed Abd El Salam[2], Eglal Hassan El Awady[3], Rania Hussien[4]

Forensic Medicine and Clinical Toxicology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

Abstract

This study aimed to assess the role of keratin 18 (K18) and high mobility group box 1 (HMGB1) biomarkers in early prediction of xenobiotics induced hepatotoxicity in comparison with ALT & AST. This is a prospective study among 50 patients presented to the PCC-ASUH with acute intoxication of hepatotoxic xenobiotics. We measured HMGB1, K18, AST and ALT on admission then patients were followed-up for development of liver injury. Subjects were classified into group I (control group), group II (no liver affection) and group III (with liver affection). ROC curve was used to compare sensitivity and specificity to report liver injury versus ALT and AST. This study showed that HMGB1 and K18 were higher in patients who developed ALI on hospital admission when ALT and AST were normal with a highly significant difference between the hepatotoxic and non-hepatotoxic group. ROC analysis showed that HMGB1 and K18 were more sensitive than ALT and AST and more specific than AST in predicting ALI. Elevations in HMGB1, and K18 identified subsequent ALI in patients on hospital admission, soon after drug and chemical overdose, while ALT and AST were normal.

Keywords: Biomarkers, hepatotoxicity, drug induced liver injury, acute liver injury

Full length article **Corresponding Author*, e-mail: dody_ali1128@yahoo.com

1. Introduction

Hepatotoxicity means chemical-driven liver damage either caused by drugs in overdoses or even when taken within therapeutic ranges, such as acetaminophen, methotrexate, depakine, iron, NSAIDs and others. In addition, chemicals may cause hepatotoxicity or natural products (e.g., microcystins) and rodenticides such as metal phosphides as well [1]. Metal phosphides are widely used rodenticides worldwide and are commercially available as dark grey powder or pellets [2]. Zinc phosphide poisoning is the most common cause of xenobiotic induced acute liver failure [3]. Worldwide, the estimated annual incidence rate of drug-induced liver injury (DILI) was 1.3-19.1 per 100,000 inhabitants and 30 percent of cases would develop jaundice [4]. Generally, liver function and injury are evaluated based upon clinical signs, serum biomarkers, and occasionally liver biopsy [5]. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are widely used parameters for diagnosis of hepatic diseases, but their half-lives in serum is relatively long, making a diagnosis more difficult especially in acute diseases. Moreover, they are non-specific as they are expressed in other organs like heart, muscle and kidney, so their serum levels increased in extra-hepatic injury, particularly in skeletal muscle [6-7]. The best liver biomarker is usually determined by many factors such as its tendency to leak during liver damage, half-life in serum as well as its specificity [7]. McGill et al., 2012 [8] suggested that high mobility group box-1 (HMGB1) and keratin-18 are sensitive biomarkers and early predictors of hepatotoxicity and that they can predict prognosis and outcome of patients intoxicated with hepatotoxic xenobiotics more than the currently used chemistry parameters; in particular the HMGB1 that has been shown to provide superior prognostic potential than ALT activity. Moreover, these new biomarkers may allow earlier

exclusion of liver injury, which would have an impact on hospital bed occupancy and also avoid adverse acetyl-cysteine reactions by reducing unnecessary treatment and permit therapy to be targeted to patients who are at high risk of adverse outcome. Measurement of these more sensitive markers alongside ALT in these patients may increase physician confidence in discharging the patient from the hospital [6]. Therefore, a prospective study was conducted on 50 adult patients of both sex presented to the PCC-ASUH with acute intoxication with hepatotoxic drugs or chemicals during the period from the beginning of June 2021 till the end of September 2022 to assess the role of K18 and HMGB1 biomarkers in early prediction of xenobiotic induced hepatotoxicity and to compare these new biomarkers with the currently used parameters (ALT & AST) in the early prediction of drugs and chemicals induced hepatotoxicity.

2. Materials and Methods

2.1. Study population

This prospective hospital based study was conducted on 50 adult patients of both sex presented to the PCC-ASUH with acute intoxication with hepatotoxic xenobiotics e.g. paracetamol, iron, metal phosphides. The diagnosis was established according to history of exposure to hepatotoxic drugs/ chemicals and the clinical characteristics. Patients who were less than 18 years old, had history of other chronic illnesses and alcoholism, pregnant females, patients presented with a delay time more than 8 hours of overdose, who take anticoagulants therapeutically or have taken an overdose of anticoagulants or who received pre-hospital treatment e.g. NAC were excluded from the study.

2.2. Subject grouping

During the study the subjects were divided into three groups; group I (control group) which included 10 healthy non-smoker volunteers (matched age and gender), group II (patients with no liver affection); included 37 intoxicated patients who were conscious and had normal vital data with no or mild symptoms and normal labs (ALT, AST, coagulation profile, random blood sugar, total and direct bilirubin, alkaline phosphatase and γGT) and group III (patients developed liver affection) which included 13 intoxicated patients with manifestations of liver affection e.g. nausea, vomiting, right hypochondrial pain, jaundice or acute liver failure and abnormal lab results e.g. elevated AST, ALT, total and direct bilirubin, coagulation profile, alkaline phosphatase and/or γGT. The Institutional Review Board of Faculty of Medicine, Ain Shams University approved the study protocol [IRB No. FMASU (MD 199/2020)]. The patients or their caregivers/family members obtained the informed consent.

2.3. Study procedures

All included patients underwent detailed history taking and clinical evaluation on arrival to ER of the PCC-ASUH and throughout the hospital stay regarding general and systemic examination for various body systems.

2.4. Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 23. The quantitative data were presented as mean, standard deviations and ranges when parametric and median, inter-quartile range (IQR) when data found non-parametric. Also qualitative variables were presented as number and percentages. The comparison between groups regarding qualitative data was done by using *Chi-square test* and/or *Fisher exact test* when the expected count in any cell found less than 5. The comparison between two independent groups with quantitative data and parametric distribution was done by using *Independent t-test* while with non parametric distribution were done by using *Mann-Whitney test.* The comparison between more than two groups regarding quantitative data and parametric distribution was done by using *One Way ANOVA test* followed by post hoc analysis using *LSD test* while with non parametric distribution was done by using *Kruskall-Wallis test* followed by post hoc analysis using *Mann-Whitney test*. *Spearman correlation coefficients* were used to assess the correlation between two quantitative parameters in the same group. *Receiver operating characteristic curve (ROC)* was used to assess the best cut off point with its sensitivity, specificity, positive predictive value, negative predictive value and area under curve (AUC) of the studied marker. The confidence interval was set to 95% and the margin of error accepted was set to 5%. So, the p-value was considered significant as the following:

- P-value > 0.05: Non significant (NS)
- P-value < 0.05: Significant (S)
- P-value < 0.01: Highly significant (HS).

3. Results and discussion

Fifty adult patients of both sex presented to the PCC-ASUH with acute intoxication with hepatotoxic xenobiotics during the period from the beginning of June 2021 till the end of September 2022. Among them, 37 patients (74%) had no liver affection (Group II) and 13 patients (26%) had liver affection (Group III). 10 healthy volunteers served as controls (Group I). The mean age of the studied patients was 24.74 ± 8.89, females (54%) from Cairo (42%) and students (46%) had the highest prevalence of exposure to hepatotoxic xenobiotics (table 1). As regards our study, aluminum phosphide was the most common ingested xenobiotic among the studied patients (46%) followed by zinc phosphide (32%) then paracetamol (22%). The median delay time before seeking medical advice was 4 hours and ranged between 1-8 hours. All patients attempted suicide orally table 2. The delay time before seeking medical advice was longer in group III than group II with a statistically significant difference with P-value 0.032 by using Mann-Whitney test (figure 1). Regarding general manifestations, pallor was more common in group III than group II with a statistical significant difference between both groups with P-value 0.020 by using Chi-square test. Jaundice occurred only in group III (46.2%) with a highly significant difference with P-value less than 0.001 by using Chi-square test. There was a statistical significant difference

between studied patients as regards shock that was found in 13.5% and 46.2% of patients in group II and III respectively. About 15% of group III had clay colored stool while all patients of group II had normal colored stool. There was a highly significant difference between both groups as regards right hypo-chondrial pain as 38.5% of patients of group III and none of group II suffered from right hypo-chondrial pain. There was a statistical significant difference between the studied groups as regards GCS as the mean GCS was 14.81 ± 0.88 and 13.85 ± 1.77 in groups II and III respectively by using independent t-test. While agitation was commonly observed in patients of group III (30.8%) with a highly significant difference by using Chi-square test (figure 3). Table (3) show that total and direct bilirubin were within normal ranges in groups I and II while they were elevated in group III with a highly statistical significant difference. INR was within normal range in groups I and II while it was elevated in group III with a highly statistical significant difference**.** Alkaline phosphatase was within normal ranges in the three groups but it was much more elevated in group III than the other groups with highly significant difference. While γGT was within normal ranges in groups I and II while it was elevated in group III with a highly statistical significant difference. Table (4) shows that the mean RBS was higher in group III than group II with a statistically significant difference between both groups.All patients had normal ALT and AST in the first day of admission, while in the second day; group III had elevated ALT and AST than group II with a highly statistical significant difference (table 5). As shown in table (6), figures (4) and (5), the newly studied biomarkers (HMGB1 and keratin 18) were much more elevated in group III than other groups with a highly statistical significant difference between the three groups. There was a highly significant increase in levels of HMGB1 and keratin 18 in group III compared with other groups with a highly significant difference (table 7).

3.1. Correlation between values of HMGB-1 and Keratin-18 in relation to other studied parameters

Highly significant positive correlations between HMGB1 on admission and keratin 18, ALT, AST in the second day of admission, total and direct bilirubin, γGT and INR were found, the higher HMGB1 the higher the values of the previous parameters. As regards keratin 18, highly significant positive correlations between keratin 18 and HMGB1, ALT, AST in the second day of admission, total and direct bilirubin, γGT, INR and PTT, the higher keratin 18 the higher the values of the previous parameters (table 8). The duration of hospital stay was longer in group III than group II with a statistical significant difference between both groups figure 6. About 92% and 45.9% of group III and II respectively were admitted to ICU with a highly significant difference. Most of patients of group II (94.6%) and group III (69.2%) recovered with a statistical significant difference between both groups figure 7. Acute liver failure (ALF) occurred in 38.5% of patients of group III. Cardiogenic shock was the cause of death in 23.1% and 2.7% of patients of group III and group II respectively and the difference was statistically significant. The second most common cause of death in patients with liver affection was acute liver failure and also the difference was statistically significant (table 9).

3.2. Predictive value of HMGB1 and keratin 18 in prediction of development of liver affection

Table (10) and Figures (8 and 9) show ROC curve analyzing sensitivity and specificity of HMGB1 and keratin 18 in predicting development of liver affection after acute intoxication with hepatotoxic xenobiotics. The cut off level of 43 ng/ml of HMGB1 achieved 92.31% sensitivity with 97.30% specificity to predict development of liver affection after acute intoxication with hepatotoxic drugs and xenobiotics while keratin 18 had 92.31% sensitivity and 97.30% specificity at cut of value 450 ng\l. In the present study, patients were divided into two groups; group II (patients with no liver affection) that included 37 (74%) intoxicated patients who were conscious and had normal vital data with no or mild symptoms and normal labs, while group III (patients developed liver affection) that included 13 (26%) intoxicated patients with manifestations of liver affection and abnormal lab results. Ten healthy non-smoker volunteers matching with age and sex served as the control group (group I). In the current study, the mean age of patients was 24.74 ± 8.89 years which is similar to results of studies done by Craig et al. (2011) [9], Antoine et al. (2013) [6] and Tan and Sklar (2017) [10], this may be attributed to several factors such as exposure of this age group to modern life stress, emotional and educational complexity, family disharmony, unemployment and financial instability, an issue that deserves particular attention. The female predominance came in agreement with Antoine et al. (2013) [6], this may be explained by female exposure to rebuke from other family members and their liability to stress more than males and as a result more prone to suicidal attempts [11-13]. The majority of patients in the current study were from Cairo (42%) followed by Qaluibeya (24%) and this could be attributed to the proximity of these governorates to the PCC-ASUH and not to the higher magnitude of poisoning problem [14] also may be due to lack of seeking medical advice by rural cases to the urban medical centers after poisoning so many cases in the rural areas are not reported to the medical authorities [15]. Students represented most of the studied patients and this was in agreement with Murari and Sharma (2014) [16], Sagah et al. (2015) [11] this may be due to admonishment from their teachers and failure or less percentages in exams. ALP was found to be the most common ingested hepatotoxic xenobiotic among the studied patients (46%) followed by zinc phosphide (32%) then paracetamol (22%)and this was in agreement with Gurjar et al. (2011) [17] stated that suicide using ALP represented about one third of the suicidal causes worldwide. The median delay time in this study was 4 (2 – 6) hours. It was increased in group III than group II. This was similar to studies done by Kariman et al. (2012) [18]; Soltaninejad et al. (2012) [19]; Elabbassi et al. (2014) [20]; Sagah et al. (2015) [11]; Dear et al. (2018) [13]. The delayed presentation in group III following overdose can explain the more adverse outcomes and the increased risk of developing multi- organ failure [21]. In agreement with previous studies done by Green et al. (2010) [22] and Remien et al. (2012) [23], the current study showed a highly significant elevation in INR in the hepatotoxic group than the other groups. Total and direct bilirubin levels on admission were within normal ranges among all studied patients then they became higher in the hepatotoxic group

(group III) than in non-hepatotoxic group (group II) and the differences were highly significant between both groups and this is in agreement with Antoine et al. (2013) [6]. The admission alkaline phosphatase was within normal ranges in the three groups but it was much more elevated in group III than the other groups with highly significant difference, in accordance with studies done by Antoine et al. (2013) [6] and Salimi et al. (2022) [24], while γGT was higher in group III while it was within normal ranges in group II with a highly statistical significant difference, in accordance with Shakoori et al. (2016) [2]. The median values of AST and ALT on admission were within the normal ranges but they were significantly higher in hepatotoxic group (group III) than non-hepatotoxic (group II). This is in accordance with studies done by Antoine et al. (2013) [6] and Dear et al. (2018) [13] who found that admission levels of ALT levels in patients who developed ALI after paracetamol toxicity were within normal ranges. This was in agreement with previous studies done by Green et al. (2010) [22] and Remien et al. (2012) [23] stated that paracetamol induced hepatocellular injury involved predominantly initial elevation of serum aminotransferases levels.

3.3. Cell death biomarkers

3.3.1. HMGB1 and keratin 18

The current study showed that HMGB1 and keratin 18 levels on admission were much more elevated in the hepatotoxic group than non-hepatotoxic group and the control group at a time that ALT and AST levels were still in normal ranges with a highly statistical significant difference between the three groups. This is in accordance with ***Dear et al. (2018)*** [13] who found that acute liver injury was predicted at hospital presentation by miR-122, HMGB1, and full-length K18. Another study done by McGill et al. (2012) [8] who involved 40 paracetamol overdosed patients and 6 healthy subjects and found elevated serum levels of K18 at the time of presentation predicting the later development of liver injury in APAP overdose patients who presented early. This also is in accordance with a study done by Antoine et al. (2012) [25] who found that total HMGB1 and K18 were significantly elevated 24 hours prior to an increase in ALT activity. Moreover, these biomarkers returned to baseline prior to ALT. Moreover, Antoine et al. (2013) [6] found normal ALT in patients with paracetamol toxicity on admission and elevated HMGB1, Apoptosis K18 and Necrosis K18 in those who developed ALI. Furthermore, significant associations between K18 and HMGB1 with poor outcome were observed [25]. Vatsalya et al. (2020) [26] found a stronger association between serum level of K18 and amount of hepatocyte death and liver disease severity than for other used biomarkers after acute alcoholic hepatotoxicity (AAH). Serum levels of K18 might be used to identify patients with severe AAH at risk for death. Passive release of HMGB1 is done by cells undergoing necrosis and acts as a Damage Associated Molecular Pattern (DAMP) molecule. HMGB1 is also actively secreted as an inflammatory mediator by inflammatory cells [26]. Davidson and Eastham (1966) [27]; Antoine et al. (2009) [28] reported that during APAP hepatotoxicity, the level of the molecular form of HMGB1 derived from necrosis was significantly increased 3-hours post-treatment, peaked at 10 hours, then decreased, and it was not detectable at 20 hours. This study also demonstrated that there was no histological evidence of further hepatocyte necrosis 15 hours post treatment, and there was evidence of liver regeneration based on the more abundant presence of mitotic hepatocytes as early as 5-hr post-treatment. This could be attributed to the fact that HMGB1 has a relatively short half-life in serum and its elevation with subsequent decrease mirrored the short-time scale of actual hepatic cell loss that highlights the importance of HMGB1 protein as a blood-based reflective indicator of pathological changes within inaccessible tissues**.** Schutte et al. (2004) [29] and Siemionow et al. (2016) [30] presented two forms of K18; full length K18 that is released from necrotic tissues and fragmented K18 that is released during apoptosis after APAP overdose. A study done by Antoine et al. (2009) showed elevation of both apoptosis and necrosis-related K18 forms following APAP toxicity. Antoine et al. (2009) reported that HMGB1 and K18 were informative serum proteins of the pathological changes induced within tissues by APAP. These proteins are related to the mechanisms of APAP-induced liver injury (apoptosis, necrosis, and inflammation) and can be used to investigate structure-metabolism relationships and structure-toxicity relationships for other hepato-toxins. As in accordance with Antoine et al. (2012), the present study showed that there were highly significant positive correlations between the cell death biomarkers (keratin 18 and HMGB1) with ALT, AST in the second day of admission, total and direct bilirubin, γGT, INR; the higher keratin 18 and HMGB1, the higher the values of the previous parameters. The current study also showed that hospital stay length and ICU admission were significantly longer in the hepatotoxic group than the non-hepatotoxic group, as in concordance with a study done by Zyoud et al., (2011a) [31]. This could be attributed to the development of liver injury and the longer need for NAC and supportive therapy in the hepatotoxic group who either presented with longer delay time or those with high risk at time of presentation (large ingested dose of xenobiotic) [32]. Receiver operating characteristic (ROC) curve analysis was done to test the sensitivity and specificity of the investigated HMGB1 and keratin 18 in predicting development of liver affection after acute intoxication with hepatotoxic drugs and xenobiotics. The cut off level of 43 ng/ml of HMGB1 achieved 92.31% sensitivity with 97.30% specificity to predict development of liver affection after acute intoxication with hepatotoxic drugs and xenobiotics while keratin 18 had 92.31% sensitivity and 97.30% specificity at cut of value 450 ng\l. This is in accordance with ***Dear et al. (2018)*** who found that HMGB1 and K18 can predict acute liver injury after paracetamol toxicity with AUC 0.94, 95% specificity and 88% sensitivity and stated that the best predictive model for prediction of acute paracetamol induced hepatotoxicity was composed of miR- 122, HMGB1, and the K18 isoforms. Beyond paracetamol overdose, Dear et al. (2018) [13] confirmed that these markers are more sensitive than current liver injury markers and recommended that they should be added to the assessment of hepatic safety for new medicines in the early phase clinical trials and showed that miR-122, HMGB1, and full-length K18 can identify acute liver injury on hospital admission at a time when currently used markers of liver injury (transaminases) were still normal.

Table 1. Socio-demographic parameters in the studied groups

		Patients group (N. = 50)	Group II (N. = 37)	Group III (N. = 13)	Test value	P-value	Sig.
Age (years)	Mean ± SD •	24.74 ± 8.89	24.16 ± 8.21	26.38 ± 10.80	0.335	0.717	NS
	Range	18 – 51	18 – 47	18 – 51			
Gender	Male	23 (46.0%)	16 (43.2%)	7 (53.8%)	4.951	0.084	NS
	Female	27 (54.0%)	21 (56.8%)	6 (46.2%)			
Residence	Cairo	21 (42.0%)	15 (40.5%)	6 (46.2%)	17.144	0.071	NS
	Fayoum	7 (14.0%)	4 (10.8%)	3 (23.1%)			
	Qaluibeya	12 (24.0%)	10 (27.0%)	2 (15.4%)			
	Giza	5 (10.0%)	5 (13.5%)	0 (0.0%)			
	Banisuef	4 (8.0%)	2 (5.4%)	2 (15.4%)			
	Asuit	1 (2.0%)	1 (2.7%)	0 (0.0%)			
Occupation	Housewife	5 (10.0%)	5 (13.5%)	0 (0.0%)	5.789	0.215	NS
	Farmer	7 (14.0%)	3 (8.1%)	4 (30.8%)			
	Student	23 (46.0%)	17 (45.9%)	6 (46.2%)			
	Employee	14 (28.0%)	11 (29.7%)	3 (23.1%)			
	Nurse	1 (2.0%)	1 (2.7%)	0 (0.0%)			

SD = standard deviation, N. = number

P-value > 0.05: Non significant

•: One Way ANOVA test

Group II: Patients with no liver affection, Group III: patients with liver affection, N.: number

Table 2. Intoxication data of the studied patients

		Patients group	Group II	Group III	Test value	P-value	Sig.
		(N. = 50)	(N. = 37)	(N. = 13)			
Type of xenobiotic	Paracetamol	11 (22.0%)	6 (16.2%)	5 (38.5%)	3.680	0.159	NS
	Aluminum phosphide	23 (46.0%)	17 (45.9%)	6 (46.2%)			
	Zinc phosphide	16 (32.0%)	14 (37.8%)	2 (15.4%)			
Delay time (hours)	Median (IQR) ≠	4 (2 – 6)	3 (2 – 5)	6 (3 – 7)	-2.149	0.032*	S
	Range	1 – 8	1 – 7	1 – 8			
Route of exposure	Ingestion	50 (100.0%)	37 (100.0%)	13 (100.0%)	–	–	–
	Inhalation	0 (0.0%)	0 (0.0%)	0 (0.0%)			
Manner of poisoning	Suicidal	50 (100.0%)	37 (100.0%)	13 (100.0%)	–	–	–
	Accidental	0 (0.0%)	0 (0.0%)	0 (0.0%)			

N.: number, IQR: inter quartile range, P-value > 0.05: Non significant; P-value < 0.05: Significant, ≠: Mann-Whitney test, N.: number, Group II: patients with no liver affection, Group III: patients with liver affection.

Table 3. Laboratory parameters among the studied.

		Group I (N. = 10)	Group II (N. = 37)	Group III (N. = 13)	Test value	P-value	Sig.
Total bilirubin (mg/dl)	Median (IQR)	0.81 (0.75 – 0.88)	0.78 (0.69 – 0.84)	1.5 (0.9 – 2)	14.170	0.001**	HS
	Range	0.7 – 0.9	0.2 – 0.93	0.4 – 7.1			
Direct bilirubin (mg/dl)	Median (IQR)	0.1 (0.09 – 0.1)	0.12 (0.1 – 0.15)	0.6 (0.5 – 0.8)	29.139	<0.001**	HS
	Range	0.08 – 0.12	0.08 – 0.2	0.1 – 5.8			
International normalized ratio (INR)	Median (IQR)	1 (0.9 – 1.02)	1.05 (1 – 1.16)	1.47 (1.23 – 2.27)	16.300	<0.001**	HS
	Range	0.8 – 1.1	0.9 – 1.3	0.9 – 5			
PTT (sec)	Median (IQR)	25 (24 – 26)	25 (23 – 29.4)	28 (25 – 38.8)	4.682	0.096	NS
	Range	22 – 30	20 – 40	22 – 120			
Alkaline phosphatase (U/L)	Mean ± SD Range	49.80 ± 5.39 42 – 60	64.16 ± 5.95 55 – 75	92.23 ± 22.64 57 – 129	42.595	<0.001**	HS
Gamma glutamyl transferase (γGT) (U/L)	Median (IQR) Range	21.5 (19 – 26) 18 – 30	39 (30 – 42) 25 – 51	160 (149 – 170) 140 – 198	41.043	<0.001**	HS

P-value > 0.05: Non significant; P-value < 0.01: Highly significant

N.: number, Group I: control group, Group II: patients with no liver affection, Group III: patients with liver affection

Table 1. Random blood sugar among the studied patients

		Group I (N. = 10)	Group II (N. = 37)	Group III (N. = 13)	Test value	P-value	Sig.
Random blood sugar (RBS) mg/dl	Mean ± SD • Range	97.70 ± 8.58 84 – 111	97.92 ± 11.00 78 – 115	108.38 ± 19.35 82 – 154	3.374	0.041*	S

P-value > 0.05: Non significant; P-value < 0.05: Significant
•: Independent t-test
N.: number, Group I: control group, Group II: patients with no liver affection, Group III: patients with liver affection

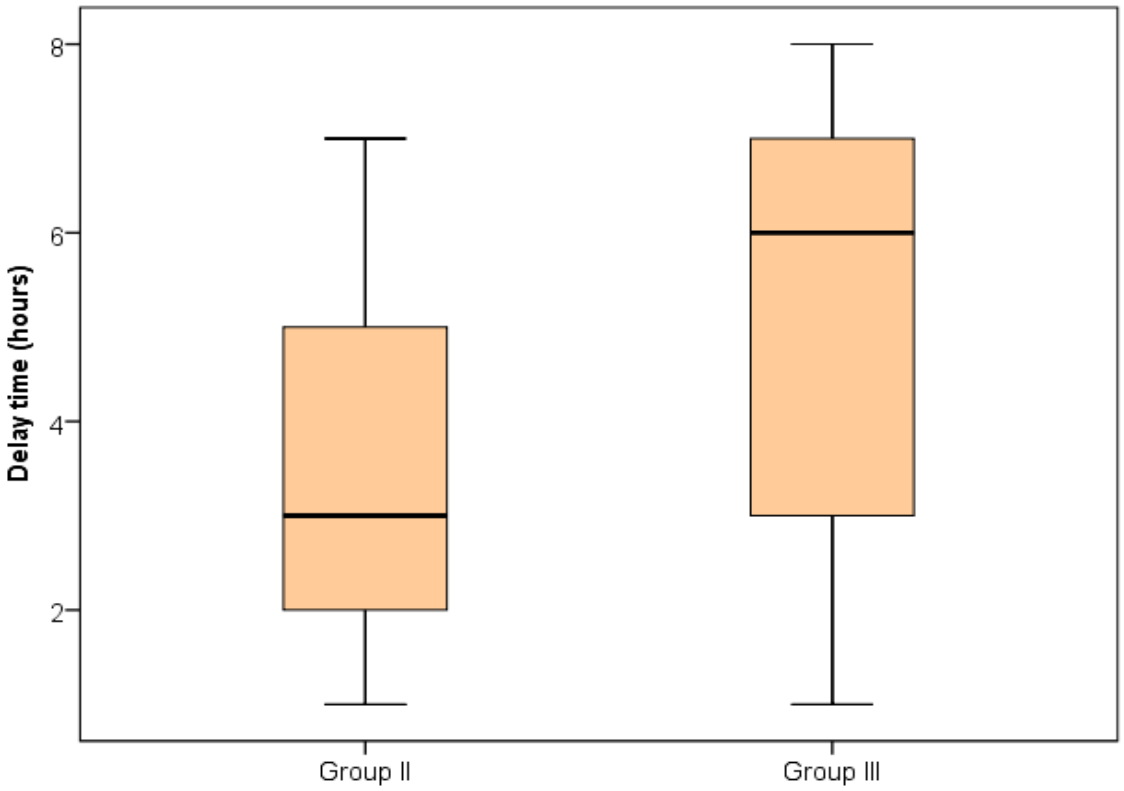

Figure 1. Box and whisker plot showing comparison between groups II and III regarding the delay time before seeking medical advice.

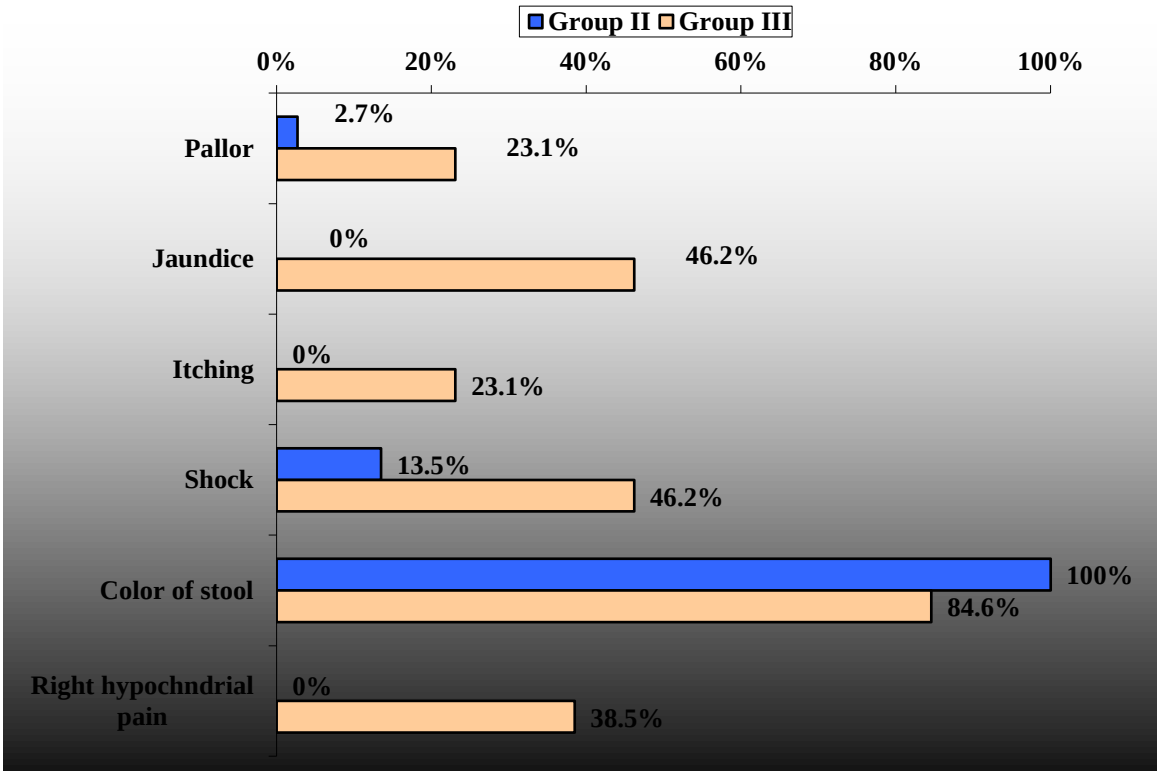

Figure 2. Histogram showing comparison between groups II and III as regards the clinical manifestations during hospital stay.

Table 5. ALT and AST in day 1 and day 2 of admission

		Studied patients (N. = 50)	Group II (N. = 37)	Group III (N. = 13)	Test value	P-value	Sig.
ALT 1 (IU/L)	Median (IQR)	13.5 (10 – 25)	13 (10 – 20)	41 (11 – 45)	-2.405	0.016*	S
	Range	4 – 383	6 – 56	4 – 383			
ALT 2 (IU/L)	Median (IQR)	19.5 (11 – 52)	14 (10 – 20)	562 (106 – 1229)	-4.965	< 0.001**	HS
	Range	8 – 5763	8 – 52	59 – 5763			
AST 1 (IU/L)	Median (IQR)	19.5 (14 – 33)	19 (14 – 22)	38 (18 – 47)	-2.104	0.035*	S
	Range	7 – 591	7 – 54	10 – 591			
AST 2 (IU/L)	Median (IQR)	21.5 (16 – 45)	18 (15 – 24)	534 (146 – 772)	-4.961	< 0.001**	HS
	Range	10 – 1896	10 – 45	59 – 1896			

P-value < 0.05: Significant; P-value < 0.01: Highly significant

N.: number, Group II: patients with no liver affection, Group III: patients with liver affection

Table 62. Cell death biomarkers (HMGB1 and keratin 18) among the studied patients.

		Group I (N. = 10)	Group II (N. = 37)	Group III (N. = 13)	Test value	P-value	Sig.
HMGB1 (ng/ml)	Median (IQR)	13.8 (12.75 – 15)	26 (22 – 30)	81 (77.2 – 88.6)	40.394	<0.001**	HS
	Range	12.2 – 17	16 – 90	27 – 98.9			
Keratin 18 (ng/l)	Median (IQR)	175.5 (165 – 181)	328 (288 – 380)	1960 (1804 – 2110)	37.760	<0.001**	HS
	Range	159 – 195	235 – 1984	222 – 2580			

P-value < 0.01: Highly significant
N.: number, Group I: control group, Group II: patients with no liver affection, Group III: patients with liver affection

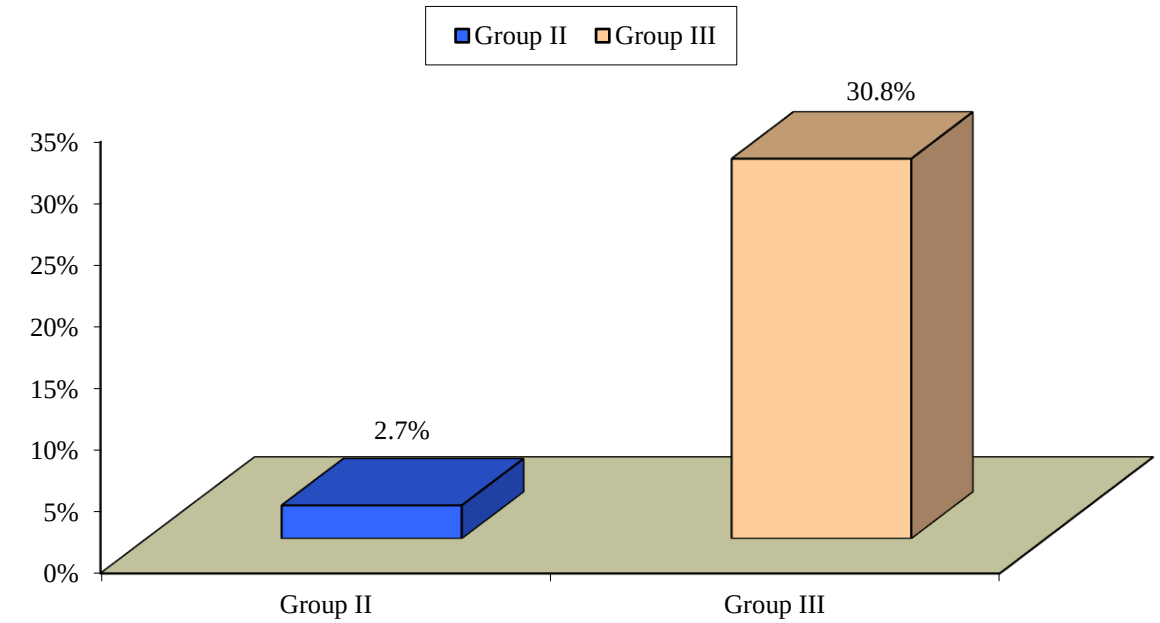

Figure 1. Histogram showing comparison between studied patients as regards agitation

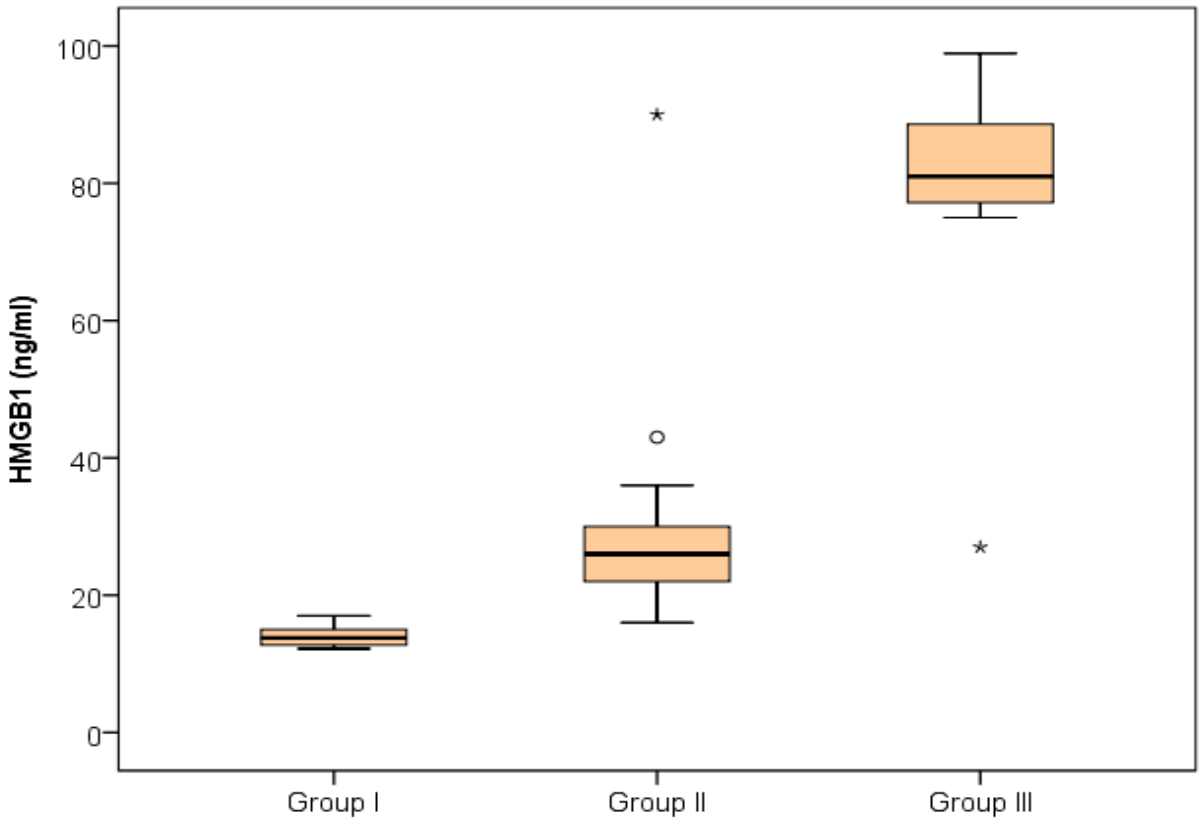

Figure 4. Box and whisker plot showing comparison between the three studied groups regarding HMGB1

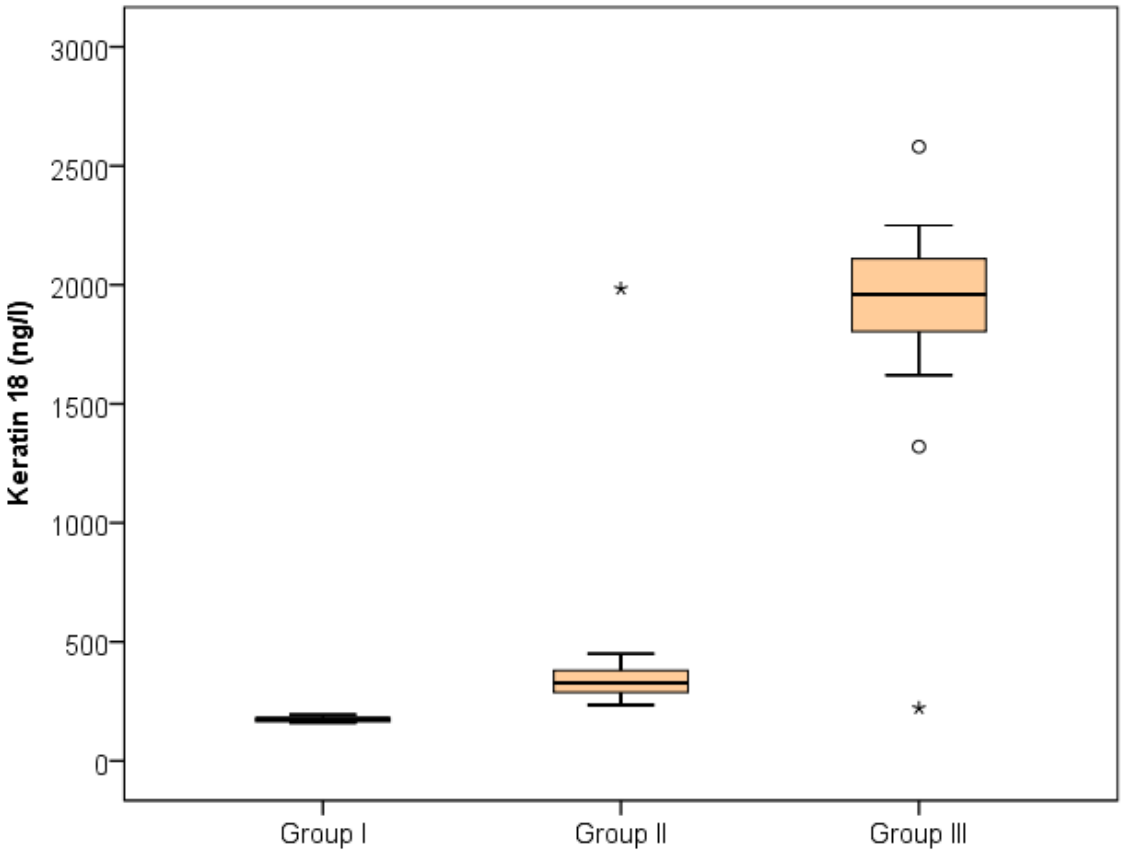

Figure 5. Box and whisker plot showing comparison between the three studied groups regarding keratin 18

Table 73. Post hoc analysis of the three studied groups regarding HMGB1 and keratin 18

	Post hoc analysis		
	Group I Vs group II	**Group I Vs group III**	**Group II Vs group III**
HMGB1 (ng/ml)	<0.001** (HS)	<0.001** (HS)	<0.001** (HS)
Keratin 18 (ng/l)	<0.001** (HS)	<0.001** (HS)	<0.001** (HS)

P-value < 0.01: Highly significant

Group I: control group, Group II: patients with no liver affection, Group III: patients with liver affection

Table 8. Spearman correlation statistical analysis of HMGB1 and keratin 18 with other studied parameters among the studied patients

	HMGB1 (ng/ml)		Keratin 18 (ng/l)	
	r	**P-value**	**r**	**P-value**
HMGB1 (ng/ml)	-	-	0.469	0.001**
Keratin 18 (ng/l)	0.469	0.001**	-	-
ALT 2 (IU/L)	0.463	0.001**	0.520	< 0.001**
AST 2 (IU/L)	0.527	< 0.001**	0.448	0.002**
Total bilirubin (mg/dl)	0.378	0.007**	0.361	0.010**
Direct bilirubin (mg/dl)	0.450	0.001**	0.437	0.002**
γGT	0.662	< 0.001**	0.540	< 0.001**
INR	0.393	0.005**	0.464	0.001**
PTT (sec)	0.173	0.230	0.390	0.005**

P-value > 0.05: Non significant; P-value < 0.01: Highly significant

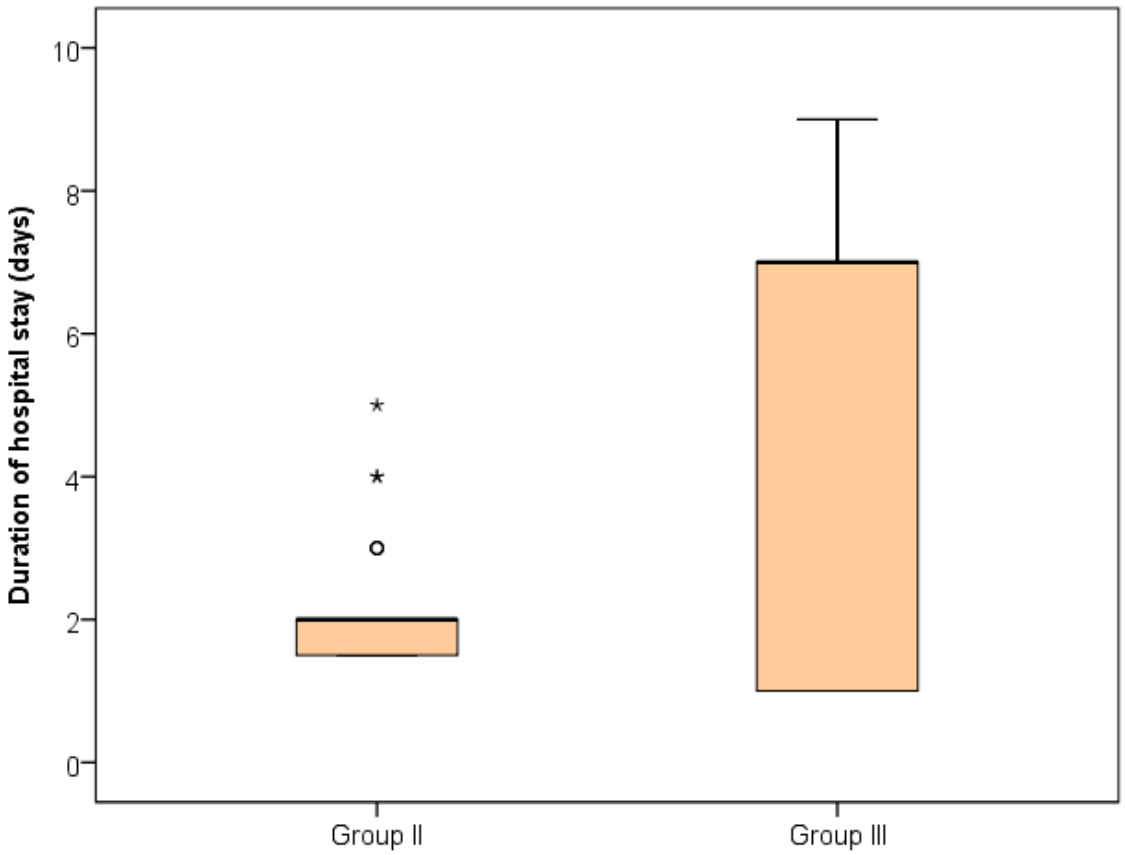

Figure 6. Box and whisker plot showing comparison between the studied patients regarding duration of hospital stay (days).

Table 9. Outcome of the studied patients

		Studied patients	Group II	Group III	Test value	P-value	Sig.
		(N. = 50)	(N. = 37)	(N. = 13)			
Duration of hospital stay (days)	Median (IQR) ≠	2 (1.5 – 3)	2 (1.5 – 2)	7 (1 – 7)	-2.060	0.039*	S
	Range	1 – 9	1.5 – 5	1 – 9			
ICU stay	Yes	29 (58.0%)	17 (45.9%)	12 (92.3%)	8.488	0.004**	HS
Duration of ICU stay (days)	Median (IQR) ≠	2 (2 – 3)	2 (2 – 2)	2.5 (1 – 4.5)	-0.336	0.737	NS
	Range	1 – 7	1 – 4	1 – 7			
Recovery	Yes	44 (88.0%)	35 (94.6%)	9 (69.2%)	5.861	0.015*	S
Acute liver failure	Yes	5 (10.0%)	0 (0.0%)	5 (38.5%)	15.812	<0.001**	HS
Mortality	Yes	6 (12.0%)	2 (5.4%)	4 (30.8%)	5.861	0.015*	S
Cause of death	Cardiogenic shock	4 (8.0%)	1 (2.7%)	3 (23.1%)	5.426	0.020*	S
	Respiratory failure	1 (2.0%)	1 (2.7%)	0 (0.0%)	0.359	0.549	NS
	Acute liver failure	2 (4.0%)	0 (0.0%)	2 (15.4%)	5.929	0.015*	S
	Renal failure	1 (2.0%)	0 (0.0%)	1 (7.7%)	2.904	0.088	NS

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.01: Highly significant

≠: Mann-Whitney test, N.: number, Group II: patients with no liver affection, Group III: patients with liver affection

Table 4. Receiver operating characteristic (ROC) curve of the diagnostic ability of high mobility group box 1 (HMGB1), keratin 18 in prediction of development of liver affection among the studied patients.

Variables	Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
ALT 1 (IU/L)	>36	0.726	69.23	97.30	90.0	90.0
AST 1 (IU/L)	>34	0.698	61.54	91.89	72.7	87.2
HMGB1 (ng/ml)	>43	0.949	92.31	97.30	92.3	97.3
Keratin 18 (ng/l)	>450	0.909	92.31	97.30	92.3	97.3

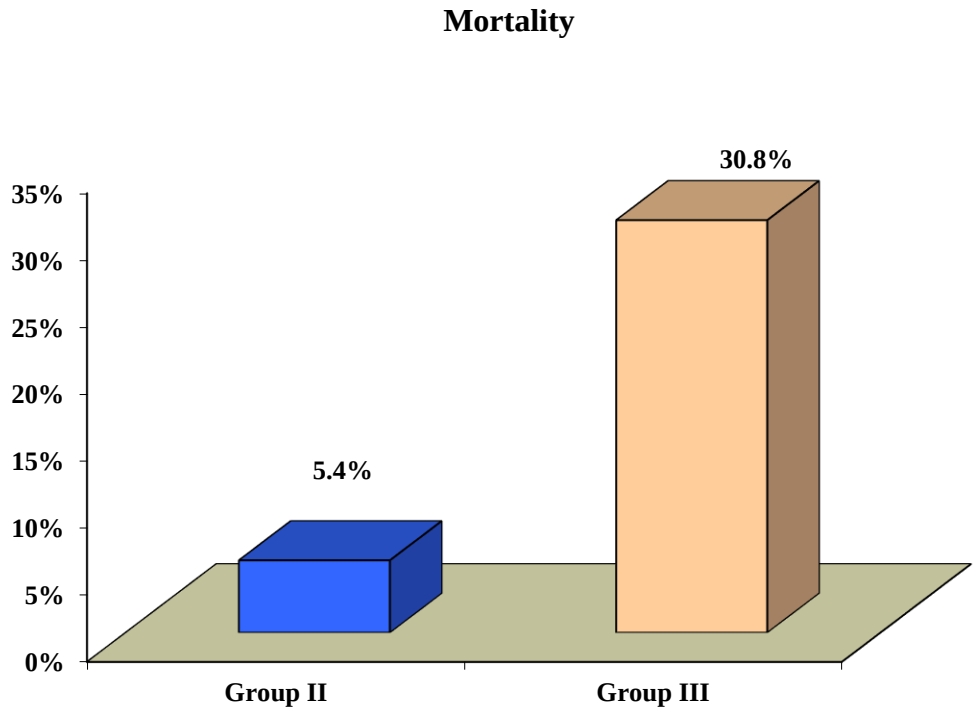

Figure 7. Histogram showing percentage of mortality among the studied patients.

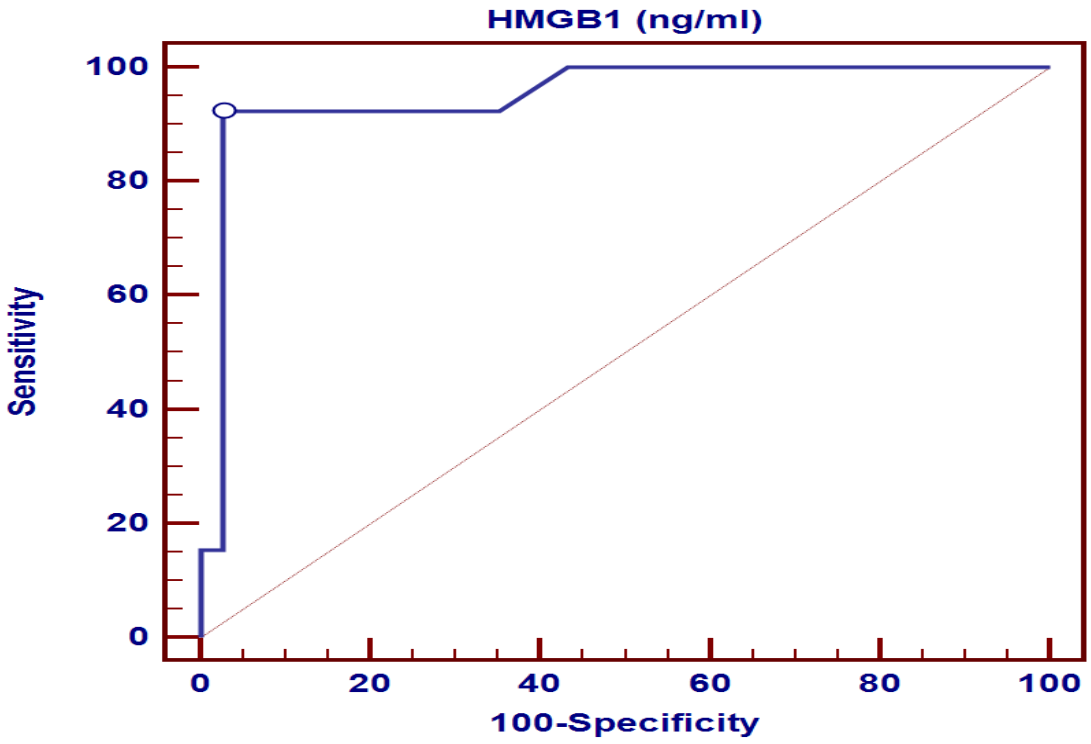

Figure 2. ROC curve of HMGB1 level for predicting development of liver affection in the studied patients.

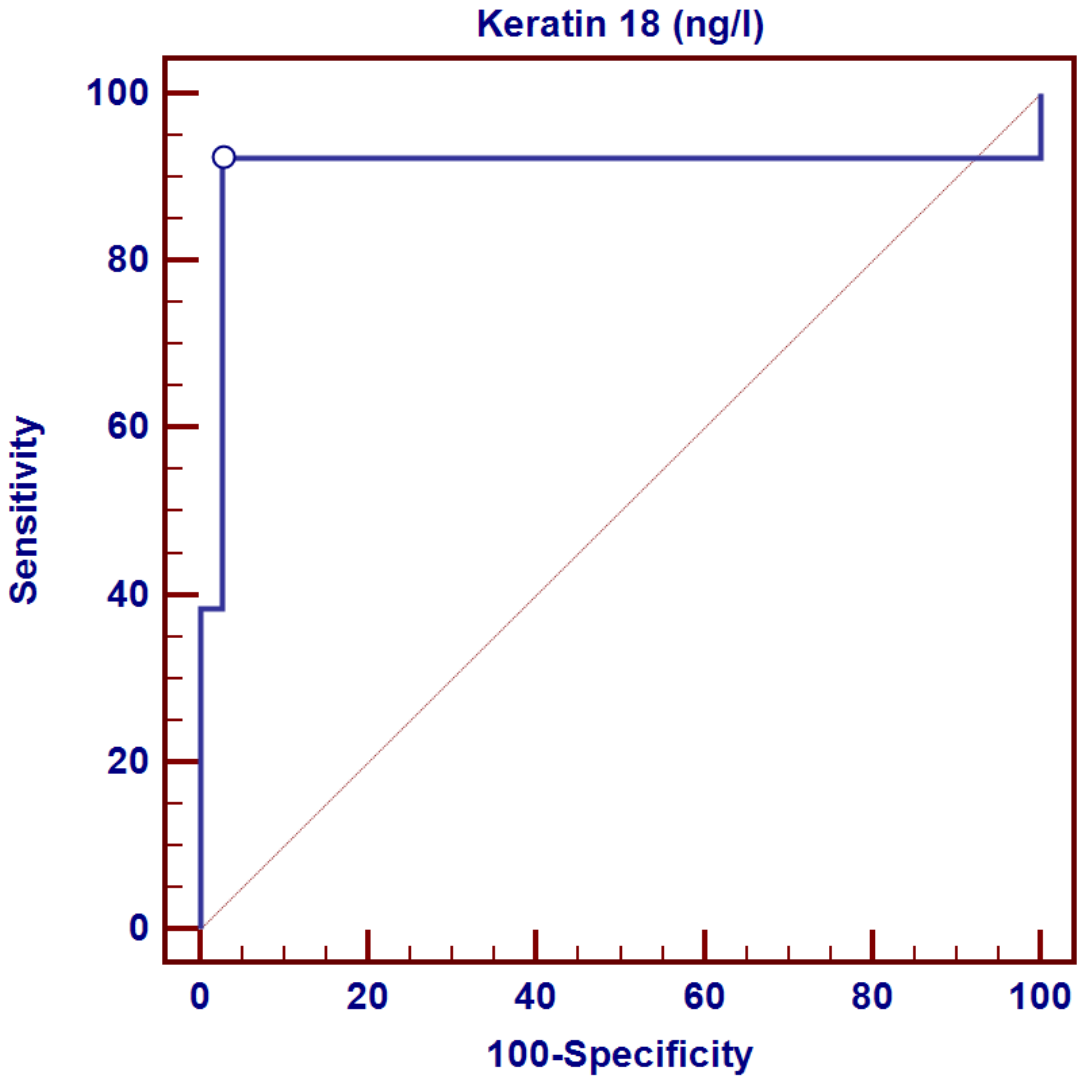

Figure 3. ROC curve of keratin 18 level for predicting development of liver affection in patients intoxicated with hepatotoxic drugs and xenobiotics.

Another study done by Antoine et al., (2009) [28] found that ROC curve analysis of the time course of APAP treatment supported the observation that K18 and HMGB1 were sensitive and specific indicators of hepatotoxicity; although ALT analysis was a more organ-specific marker of hepatic damage. Antoine et al., (2013) [6], stated that HMGB1 can predict acute liver injury after paracetamol toxicity with AUC 0.97, 91% specificity and 91% sensitivity while K18 can predict acute liver injury with AUC 0.94, 90% specificity and 87% sensitivity. While ALT activity on admission was a poor predictor of the development of acute liver injury, with an AUC of 0.54 and a sensitivity of 0.09 at 90% specificity.

3.4. Limitations

We can identify some limitations in our study. Firstly, the current study is a single-center study; therefore, the findings from our study may not be generalizable to all patient populations with ingestion of hepatotoxic xenobiotics. secondly, patients younger than 18 years were not included in the study, making it impossible to evaluate the role of cell death biomarkers in prediction of liver injury. Therefore, additional multicenter are required to determine the prognostic usefulness of HMGB1 and K18 and their association with the emergence of liver affection after ingestion of hepatotoxic xenobiotics.

4. Conclusions

In conclusion, plasma HMGB1and K18 levels on admission are useful early predictive biomarkers for diagnosis of acute drug induced hepatotoxicity and are more sensitive than ALT in identifying drug- induced acute liver injury. HMGB1 and keratin 18 may allow earlier exclusion of injury, which would have an impact on hospital bed

occupancy and avoid adverse acetyl cysteine reactions by reducing unnecessary treatment, but further studies are required.

Disclosure statement

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

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