

Predictive Value of Non-Invasive Blood Ammonia Level for the Presence of Oesophageal Varices in Egyptian Patients With Liver Cirrhosis

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ABSTRACT

AIM: Although recent guidelines recommend screening of cirrhotic patients by upper endoscopy for oesophageal varices prediction, Non-invasive parameters are needed due to high endoscopy cost and burden on endoscopic units particularly in poor countries. The aim of this study is to evaluate the accuracy of using ammonia blood level as potential non-invasive predictor of oesophageal varices in cirrhotic patients.

METHODS: This prospective study was conducted on 150 Egyptian participants. Patients were categorized as group I which included 100 with oesophageal varices and group II included 30 patients without varices. There were 20 healthy control participants served as a control group (group III). All patients underwent for full clinical and laboratory workup, abdominal ultrasound and upper gastrointestinal endoscopy. Venous ammonia blood levels were calculated for all the contributors of this study.

RESULTS: The mean ammonia level was higher in group I (88.29 ± 42.82 $\mu\text{mol/L}$) than in group II (82.77 ± 49.76 $\mu\text{mol/L}$) with

no statistical significant difference between the two groups ($p=0.34$). Ammonia level in group III was 73.37 ± 30.36 $\mu\text{mol/L}$ with no statistical significant difference with the other groups ($p>0.05$). In group I; ammonia level is positively correlated with the splenic vein diameter with $r = 0.22$ ($p=0.026$) but did not correlate with the grade of oesophageal varices $r = 0.031$ ($p\text{-value}=0.762$). In multivariate analysis; ammonia combined with platelets, Age, PT and PC shared in a significant prediction model (I) for esophageal varices grading ($p=0.002$). Prediction model (II) including portal and splenic veins diameters and the liver size was developed ($p=0.016$).

CONCLUSION: Non-invasive means could be used to monitor cirrhotic patients and consider treatment. Ammonia level can not be used alone but its use within a significant prediction model can help restricting the use of endoscopic screening in patients with a high probability of esophageal varices.

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Key words: Non-invasive; Varices; Ammonia; Prediction model; Cirrhosis

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INTRODUCTION

Oesophageal varices due to portal hypertension are a major concern in cirrhotic patients because of the risk of bleeding^[1]. The prevalence of oesophageal varices in newly diagnosed cirrhotic patients is approximately 60-80% and the 1-year rate of first variceal bleeding is approximately 5% for small oesophageal varices and 15% for

large oesophageal varices^[2]. The determination of the presence of oesophageal varices by upper digestive endoscopy is therefore mandatory in patients with cirrhosis at diagnosis^[3].

For long-term follow up, guidelines recommend monitoring of cirrhotic patients by routine endoscopy for the detection of the development of oesophageal varices and to initiate prophylactic measures to prevent the bleeding of oesophageal varices when they become large^[4]. Endoscopy is however a costly, invasive, and time-consuming procedure^[5]. In Egypt; chronic liver diseases are common due to the higher prevalence of viral hepatitis C and Schistosomiasis^[6].

In cirrhosis the main portion of blood ammonia carried by portal blood is shunted by portosystemic shunts to systemic circulation could be a good mirror of portosystemic collaterals and consequently portal hypertension^[7]. The aim of this study is to evaluate the accuracy of using ammonia blood level as potential noninvasive predictor of esophageal varices in cirrhotic patients.

METHODS

This study was conducted on 130 adult Egyptian patients with HCV related liver cirrhosis from August 2011 to March 2012. Patients were assigned into: group I including 100 patients with esophageal varices documented by upper endoscopy. Group II including 30 patients without varices. Both groups were recruited from Endemic Medicine Department, Cairo University. Group III included 20 participants seronegative for HCV and HBV who were included in our study to serve as a control group. Written informed consents were obtained from all participants and the study protocol was approved by our institute ethical committee. Pregnant and lactating females, other causes of cirrhosis than HCV, Patients with hepatocellular carcinoma or portal vein thrombosis and previous variceal ligation or injection sclerotherapy were excluded from the start.

All patients were subjected to Full history taking and clinical examination. The following investigations had been done: Complete liver & kidney functions, coagulation profile, complete blood picture, ESR, HCV antibodies, HBsAg and HbC Ab. Abdominal ultrasound was performed to confirm liver cirrhosis, assess the spleen size

and detect the presence of ascites. All patients underwent an upper gastrointestinal endoscopy. Grading classification of I - IV was used^[8].

Ammonia determination

For the quantitative determination of Ammonia in plasma an Enzymatic UV-Method manual (RANDOX LABORATORIES LTD. Ardmore, Diamond Road, Crumlin, Co. Antrim, United Kingdom BT29 4 QY) was done^[9].

Statistical analysis

Patients' data were analyzed using SAS 9.2 for windows. Quantitative variables were expressed by mean and SD (Standard deviation), compared using t-student test and Mann-Whitney test were used when appropriate. Qualitative variables were expressed by numbers (Frequency) and percent. Proportions were analyzed using Fisher's exact test. Prediction model were performed for calculating predicted probabilities. P- Value was considered to be significant if less than 0.05.

RESULTS

One hundred and thirty patients with HCV related liver cirrhosis were enrolled and divided into 2 groups according to the presence or absence of oesophageal varices of 100 and 30 patients respectively; in addition to group III of 20 healthy control participants. The epidemiological data revealed that, age was found statistically higher in patients of group II (51.50±11.02 years) as compared to those of group I (50.17±12.89 years) and group III (44.70±11.51years) and this is not significant.

There was no statistical significant difference among the studied groups regarding their sex distribution (group I included 62 males and 38 females, group II included 22 males and 8 females and group III included 12 males and 8 females) where the p- value was 0.2 & 0.8 on comparing withgroup I. The laboratory and ultrasonographic showed with no significant difference between group I and group II. The controls were normal in all their laboratory study. Data of the studied groups are shown in table 1.

Table 1 Laboratory and Ultrasonographic findings for the studied patients.

Parameters	Group I (n=100) Mean ± SD	Group II (n=30) Mean ± SD	P value GI, GII
Age in years	18 - 75 (50.17 ± 12.89)	19 - 65 (51.50 ± 11.02)	25 - 63 (44.70 ± 11.51)
Gender Male/Female	62/38	22/8	12/8
Total bilirubin: (0,1-1,0mg/dL)	2.99 ± 3.47	4.00± 4.99	0.38
HB(M: 12,5 - 15,5g/dl) (F:11,5- 14,5g/dL)	9.88 ± 2.15	10.80 ± 2.04	0.04
Platelets: (n=150000-450000/dL)	138270.00 ± 91315.50	150233.33 ± 96588.24	0.6
AST (0-38 µ/L)	68.57 ± 57.18	59.30 ± 32.65	0.45
ALT (0-40) µ/L	44.55 ± 32.83	47.96 ± 45.24	0.9
Albumin (3,5-5,5) g/dL	2.49±0.85	2.62 ± 1.01	0.69
INR	1.59 ± 0.38	1.47 ± 0.33	0.17
Creatinine (0.6-1.3mg/dL)	1.29 ± 0.88	1.15 ± 0.50	0.78
Portal vein (mm): Mean ± SD	13.44± 3.26	13.94 ± 4.38	< 0.05
Splenic size:			
• Normal (<12cm)	9 (9%)	7 (23.3%)	
• Mild splenomegaly(13-16cm)	55 (55%)	8 (26.6%)	
• Moderate splenomegaly(16-20 cm)	36 (36%)	13 (43.3%)	
• Huge splenomegaly(>20cm) ^[10]	0 (0%)	2 (6.6%)	
Splenic vein (mm):	12.96 ± 1.89	13.14 ± 1.99	< 0.05
Ascites:			
No	10 (10%)	5 (16.7%)	
Minimal	17 (17%)	6 (20%)	
Moderate	48 (48%)	13 (43.3)	
Massive	25 (25%)	6 (20%)	

P < 0.05 is significant.

Comparing the venous ammonia level among the 3 groups; The mean ammonia level was higher in group I (88.29±42.82 µmol/L) than in group II (82.77± 49.76 µmol/l) but with no statistical significant difference between the two groups (*p*-value = 0.34). Ammonia level in group III was 73.37±30.36 µmol/L with no statistical significant difference with the other groups (*p*-value <0.05).

When we categorized group I according to the degree of esophageal varices (I-IV) there was no statistical significant difference in venous ammonia level between patients with the different grades of esophageal varices (Table 2). With further division of group I into two subgroups IA and IB according to the varices grade into: Group IA (*N*=65) = esophageal varices grades I, II (small varices) and Group IB (*N* =35) = esophageal varices grades III, IV (medium and large varices). Patients of group IB have higher mean ammonia level (91.12±47.53) than those of group IA (86.76±40.36) but this difference is not significant with *p*- value = 0.63.

Among patients of group I (*n*=100) we found that the ammonia level is positively correlated with the splenic and portal veins diameters with *p*=0.026 and 0.001 respectively, but did not correlate with the grade of oesophageal varices *p*=0.762 0.231 respectively. An inverse correlation was observed i.e., *r* = -0.341 (*p*=0.001) between the splenic size and the platelet count (Table 3).

By applying a prediction model (I) in patients of group I it was found that ammonia alone is not a significant predictor to the grade of the oesophageal varices, but in multivariate analysis it was found that ammonia shares in a significant prediction model for predicting the grade of oesophageal varices and this model is statistically significant with *p*- value= 0.002, where platelets, Age, PT and PC became a significant model for prediction of the grade of oesophageal varices (grade I, grade II, grade III or grade IV) with *p*-values 0.038, 0.040, 0.001, 0.001 respectively which were highly significant (Table 4).

Table 2 Comparison between ammonia levels in different grades of oesophageal varices in group I.

Variable tested	Oesophageal varices grades			P value
	N	Mean±SD		
Ammonia (10-47 µmol/L)	Grade I	36	88.89±37.18	0.123
	Grade II	29	81.56±46.07	
	Grade III	32	103.64±42.07	
	Grade IV	3	65.22±68.98	
	Total	100		

P< 0.05 is significant.

Table 3 Correlations in the oesophageal varices group (group I, *N*=100).

	Oesophageal varices	NH4	Platelets	Portal vien	Spleen size	Splenic Vein
NH4						
R	0.031		0.068		0.105	0.223*
p-value	0.762		0.499		0.296	0.026
N	100		100		100	100
Platelets						
R	-0.179	0.068		0.165	-0.341**	-0.175
P-value	0.075	0.499		0.080	0.001	0.081
N	100	100		100	100	100
Portal						
vein R	0.121	0.213*			0.091	0.470**
P-value	0.231	0.025			0.284	0.001
N	100	100			100	100
Spleen size						
R	0.019	0.105	-0.341**	0.091		0.480**
P-value	0.852	0.296	0.001	0.284		0.0001
N	100	100	100	100		100
Splenic						
vein R	0.122	0.223*	-0.175	0.470**	0.480**	
P-value	0.228	0.026	0.081	0.001	0.0001	
N	100	100	100	100	100	

* Correlation is significant at *P* <0.05; ** Correlation is highly significant at *p* <0.01.

Prediction model (2) replacing platelets and age in the previous model with portal vein diameter and liver size assessed using ultrasonography and adding group II cirrhotic patients without varices to group I (*N*= 130). Prediction of oesophageal varices grade using our second model is significant with *p*- value = 0.016 (Table 5).

Table 4 Prediction model (I) including NH4, platelets, age, PT, PC, and RBC for prediction of oesophageal varices.

Prediction model	Estimate risk (Odds ratios)	95% confidence interval		P value
		Lower bound	Upper bound	
Oesophageal				
(grades = 1)	-10.296	-16.650	-3.943	0.001*
Varices				
(grades = 2)	-8.165	-14.427	-1.904	0.011*
(grades = 3)	-5.083	-11.089	0.924	0.097
Parameters:				
platelets	-5.429E-6	-1.055E-5	-3.084E-7	0.038*
age	0.035	0.002	0.068	0.040*
PT	-0.302	-0.485	-0.119	0.001*
PC	-0.081	-0.129	-0.032	0.001*
NH4	0.002	-0.007	0.011	0.613
RBC	-0.269	-0.835	0.297	0.351

P< 0.05 is significant.

Table 5 Prediction model (II) including NH4, PC, PT, RBC, PV, liver size for prediction of oesophageal varices.

		Estimate risk (Odds ratios)	95% confidence interval		P value
			Lower bound	Upper bound	
Oesophageal varices	[grade = 0]	-8.984	-13.941	-4.027	0.0001*
	[grade = 1]	-7.617	-12.520	-2.715	0.002*
	[grade = 2]	-5.909	-10.745	-1.074	0.017*
	[grade = 3]	-3.266	-8.158	1.625	0.191
	PT	-0.162	-0.306	-0.018	0.028*
Parameter	PC	-0.047	-0.080	-0.014	0.006*
	RBC	-0.598	-1.070	-0.125	0.013*
	NH4	0.001	-0.007	0.008	0.843
	PV	-0.033	-0.124	0.058	0.479
	[LiverSize=average]	0.693	0.017	1.369	0.045*
	[LiverSize=enlarged]	-0.003	-1.364	1.358	0.997
	[LiverSize=shrunken]	0 ^a	.	.	.

P< 0.05 is significant.

DISCUSSION

Portal hypertension contributes slowly and constantly to hepatic insufficiency^[11]. Esophageal varices are the most critical portosystemic shunts that develop secondary to portal hypertension. Endoscopic prophylactic band ligation and non-selective beta blockers can reduce the risk of oesophageal bleeding by 50%^[12]. Endoscopic screening of all cirrhotic patients would lead to a large number of unnecessary endoscopies and additional burden to endoscopic units^[13]. Several studies have discussed how to identify patients with varices using non-invasive or minimally invasive methods^[14]. The development of non-invasive methods for oesophageal varices prediction could reduce the use of upper gastrointestinal endoscopy in variceal screening and also provide an alternative way to confirm the results of conventional endoscopic diagnosis^[15].

Concerning our study; As regards the epidemiological features of our patients; their age range from 18-75 years in agreement with the studies done by Cherian *et al* 2011^[16] and Serag *et al* 2011^[17] who reported that the age peak of cirrhotic patients was at the fifth decade. Males were more than females in agreement with Cherian *et al*, 2011^[16] as they found that the incidence of infection was more predominant in male due to higher probability of viral exposure. All our cirrhotic patients were caused by HCV as Egypt has the highest prevalence of HCV worldwide (15%)^[18] with genotype-4a accounting for almost 90% of infections. Egypt has an estimated annual incidence of HCV about 150,000 cases^[19].

In our study; the mean ammonia level was higher in patients with oesophageal varices than in those without varices and controls. In addition, ammonia level was also higher in patients with higher grades of varices but with no statistical significant difference between the groups thus we can conclude that blood ammonia level can not differentiate between any of the studied groups although this disagree with Tarantino *et al* 2009^[11] and Khondaker *et al* 2013^[7]. To explain the relatively high ammonia level in our control group; as matter of fact, the blood ammonia determination suffers from some limits in its measurements. In fact, the collection, handling, storage, and analysis of blood samples are all potential sources of error. Anyway, recommendations ought to be made on the collection and processing of blood samples, for it is by standardization and rigid adherence to these techniques that the reliability of the test results will be improved^[11].

Our continued analysis of blood ammonia concentrations highlighted a significant correlation between them and splenic vein diameter with no correlation existed with platelet count or splenic longitudinal length and this also disagree with Tarantino *et al*^[11] who reported significant correlation of ammonia levels with platelet count but not with ALT or spleen diameter.

From all the above we concluded that ammonia alone can not predict the presence nor the grade of oesophageal varices. So, we put ammonia in a model with platelets, age, prothrombin time, prothrombin concentration, RBCs by applying this model in patients of group I we found that ammonia alone is not a significant predictor to the grade of esophageal varices, but in multivariate analysis it was found that ammonia shared in a significant prediction model for predicting the grade of oesophageal varices (prediction model I). By studying patients in both in groups (I&II) using another prediction model (prediction model II) that replace platelets, and age in the previous model with, RBCs; portal vein diameter and liver size by ultrasonography it was found that ammonia is not a significant predictor for the presence of esophageal varices ,but on multivariate

analysis it was reported that ammonia shares in a significance with these prediction models. This is in agreement with Tarantino *et al*^[11] who found that blood ammonia concentrations can predict the portosystemic veins by using the PLTs and SLD. Study that was done by Eslam *et al* 2013^[20] who showed that presence of oesophageal varices was independently associated with lower platelet count low platelet count and advanced Child-Pugh score. Wang *et al* in 2014 showed that hemoglobin level, portal vein diameter and the ratio of platelet count/spleen diameter contributed significantly in univariate analysis for prediction of large varices.

In conclusion; despite the lower diagnostic accuracy of the models, but this models were good to be used as part of tools to monitor cirrhotic patients and consider treatment. However, upper digestive endoscopy remains the more reliable means to monitor cirrhotic patients.

CONCLUSION

Despite the lower diagnostic accuracy of the models, but this models were good to be used as part of tools to monitor cirrhotic patients and consider treatment. However, upper digestive endoscopy remains the more reliable means to monitor cirrhotic patients.

CONFLICT OF INTERESTS

There are no conflicts of interest with regard to the present study.

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