Study of the Relationship between Blood Ammonia Level and Esophageal Varices in Patients with Liver Cirrhosis

Atef Abo-Alsoud Ali [1], Amira Maher Badawy [1], Ahmed Abdel-Rahman Sonbol [2], Mohammed Elsayed Ayad [3]

[1]*Tropical Medicine department, Faculty of Medicine, Menoufiya University ,Egypt*
[2]*Clinical Pathology department, Faculty of Medicine, Menoufiya University,Egypt*
[3]*Shebin Elkom Teaching Hospital ,Shebin Elkom ,Egypt*

Corresponding Author
Mohamed Elsayed Ayad

Mobile:
01063382104

E mail:
Drayad92@gmail.com

Key words:
Oesophageal varices, Portosystemic collaterals, Blood ammonia, Upper gastrointestinal endoscopy

Background and study aim: Portal hypertension leads to formation of portosystemic collateral veins as oesophageal varices in patients with liver cirrhosis. Several studies have shown that blood ammonia is a vauable non invasive marker of oesophageal varices. This study is designed to evaluate the possible relation between blood ammonia level and oesophageal varices in patients with liver cirrhosis.

Patients and Methods: This study was conducted on three groups of patients and control subjects. Group I included 20 cirrhotic patients without evidence of oesophageal varices. Group II included 40 cirrhotic patients with evidence of varices. Group III included 20 healthy control subjects. Serum level of ammonia were done for all patients and control subjects.

Results: There was a highly significant increase in the mean values of blood ammonia in cirrhotic patients with varices in comparison to other patients without varices, with highly significant positive correlation between serum ammonia and size of varices. There was a significant increase in the mean values of blood ammonia in cirrhotic patients with grade III and IV varices [large varices) in comparison to cirrhotic patients with grade I and II varices.

Conclusions: Blood ammonia level is a valuable, simple non invasive marker for prediction of oesophageal varices.

INTRODUCTION

Cirrhosis is defined histologically as a diffuse hepatic process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules. The progression of liver injury to cirrhosis may occur over weeks to years. Indeed, patients with hepatitis C may have chronic hepatitis for as long as 40 years before progressing to cirrhosis **[1].**

Portal hypertension is a progressive, inevitable consequence of liver cirrhosis, which leads to formation of porto-systemic collateral veins. Among them, oesophageal varices have the greatest clinical impact because their rupture results in variceal hemorrhage that can be fatal. Upper gastrointestinal [GI) endoscopy is the gold standard in the diagnosis of oesophageal varices **[2]**.

Gastroesophageal varices are present in approximately 50% of patients with liver cirrhosis. Their presence correlates with the severity of liver disease. Patients without varices develop them at a rate of 8% per year and the progression from small to large varices occurs in 10 to 20% of cases yearly **[3]**. The most important predictor of hemorrhage is the size of varices, with the highest risk of first hemorrhage occurring in patients with large varices, about 15% per year **[4]**.

Practice guidelines have recommended that all patients with cirrhosis undergo screening upper GI endoscopy to detect oesophageal varices at the time of diagnosis and after that, surveillance endoscopies should be performed every 2 to 3 years in cirrhotic patients without varices and the patients with small varices be endoscoped every 1-2 years, and annually in the setting of decompensation **[5]**. However, these guidelines have not been evaluated prospectively to date ,particularly regarding its cost effectiveness. Since, the point prevalence of medium/large

varices is approximately 15 to 20% **[6]**, the majority of subjects undergoing screening endoscopy either do not have varices or have varices and do not require prophylactic therapy.

Therefore, there is need for identification of non endoscopic, non invasive methods that can accurately predict oesophageal varices, particularly large ones in cirrhotic patients and help to identify patients at greatest risk and thereby reduce the necessity of endoscopic screening **[7]**.

In cirrhosis, the major portion of ammonia carried by portal blood is shunted by portosystemic collaterals into systemic circulation. The raised blood ammonia level may be an indicator of the presence of oesophageal varices as a main part of portosystemic shunts with the most dangerous complications **[8)**.

On the other hand, a recent study found that there was a moderate but significant correlation between blood ammonia level and size of oesophageal varices **[9]**.

PATIENTS AND METHODS

This study was carried out on patients attending the outpatient clinic and the inpatients of Hepatology and Gastroenterology unit of Shebin Elkom Teaching Hospital in the period between June to December 2014, from which 60 cirrhotic patients were selected, in addition to 20 healthy persons of matched age and sex as controls. The cirrhotic patients were further subdivided into the two groups. Group I included 20 patients without endoscopic evidence of oesophageal varices, Group II included 40 patients with endoscopic evidence of oesophageal varices, this group was subdivided into 4 subgroups according to grade of varices, Group IIa included 10 patients with grade I oesophageal varices, group IIb included 10 patients with grade II oesophageal varices, group IIc included 10 patients with grade III oesophageal varices and group IId included 10 patients with grade IV oesophageal varices. After having an informed consent, all patients and controls were subjected to full history taking, full clinical examination, abdominal ultrasonography, upper gastrointestinal endoscopy and laboratory investigations including: CBC, ESR, RBS, liver function tests, AFP, creatinine & BUN, serum electrolytes, HCV Ab & HBs Ag and serum ammonia were done for all patients.

Exclusion criteria

Patients who received endoscopic variceal ligation (EVL) or sclerotherapy, presence of hepatic encephalopathy, active or recent GI bleeding within 4 weeks, portal vein thrombosis on ultrasonography [USG), hepatocellular carcinoma, renal insufficiency evidenced by serum creatinine of >1.3 mg/dl and patients in whom endoscopy is contraindicated.

Sample collection and measurement of ammonia

Fasting blood ammonia level was measured in all patients and controls within 1 to 3 days of performing endoscopy. Patients were asked to fast overnight. In the morning, at complete rest, 5 ml of peripheral venous blood was taken from each subject without using tourniquet. Blood was collected into an EDTA evacuated tube. The samples were immediately carried to laboratory gently in an icebox and analyzed within 30 minutes of arrival. In cases of ambulant patient, samples were collected in the laboratory. During analysis, sample was first centrifuged and the plasma was separated from cellular material. Ammonia level was measured by kinetic enzymatic method with glutamate dehydrogenase by using ammonia–liquizyme single reagent provided by Bioassay system, Germany.

Statistical Analysis :

All the patient details and study variables were entered in predesigned data collection sheet. Data were analyzed by using statistical software SPSS 13.0. All the quantitative data were expressed as mean ± SD; qualitative data were analyzed by Chi-square test and quantitative data by Student's t-test or Mann-Whitney's U test. Correlation study was done by using Spearman's correlation coefficient test. Performance of the test was assessed by sensitivity and specificity. Receiver operating characteristic [ROC) curve was used to assess the usefulness of the test and performance at different cutoff values. A 'p' value of <0.05 was taken statistically significant and a 'p' value of <0.005 was taken highly statistically significant.

RESULTS

There was a high statistically significant difference between the mean values of blood ammonia level of cirrhotic patients and control group as shown in table (1).

There was a high statistically significant increase in the mean values of blood ammonia in cirrhotic patients with varices in comparison to other patients without varices as shown in table (1).

The best cutoff value for detection of O.V. was 74 umol/L with sensitivity 97.5%, specificity 80%, positive predictive value (PPV) 90.7% and negative predictive value (NPV) 94.1% as shown in table (3) and figure (1).

On the other hand, there was a high statistically significant decrease in the mean values of platelet count/ spleen diameter ratio in cirrhotic patients with varices in comparison to other patients without varices as shown in table (2). The best cutoff value for detection of O.V was 638.9 with sensitivity 100%, specificity 97.5%, PPV 95.2% and NPV 100% as shown in table (3).

There was a high statistically significant positive correlation between blood ammonia and size of varices as shown in table (4) and figure (2), while there was a high statistically significant negative correlation between platelet count/ spleen diameter ratio and size of varices as shown in table (4).

There was a statistically significant decrease in the mean values of platelet count/spleen diameter ratio in cirrhotic patients with evidence of grade III and IV varices (large varices) in comparison to cirrhotic patients with grade I and II varices as shown in table (5). The best cutoff value for detection of large varices was 436.5 with sensitivity 82.5%, specificity 70%, PPV 84.6% and NPV 66.7% as shown in table (6).

There was a statistically significant increase in the mean values of blood ammonia in cirrhotic patients with evidence of grade III and IV varices (large varices) in comparison to cirrhotic patients with grade I and II varices as shown in table (5). The best cutoff value was 102 umol/L with sensitivity of 95% and specificity of 52.5% in detecting large esophageal varices (grade III and IV varices) in patients with liver cirrhosis. Its PPV was 50% and NPV was 95.5% with accuracy of 66.7% as shown in table (6) and figure (3).

Table (1): Blood ammonia level in the studied groups

	Cirrhotic group with varices (no=40)	**Cirrhotic group without varices (no=20)**	**Total cirrhotic patients (no=60)**	**Control group (no=20)**	**t-Test**	**P value**
	Mean ± SD		**Mean ± SD**	**Mean ± SD**		
Ammonia (umol / L)	135.1±24.8	65.8±11.3	111.9±39.1	45.5±4.4	t1:9.14 t2: 11.87	p1<0.001** p2<0.001**

**Highly significant difference
t1 and p1: between total cirrhotic patients and control group.
t2 and p2 : between cirrhotic patients with and without varices.

Table (2): Platelet count /spleen diameter ratio in cirrhotic patients

	Cirrhotic group with varices (group II) [no=40)	**Cirrhotic group without varices (group I) (no=20)**	**t-Test**	**P value**
	Mean ± SD	**Mean ± SD**		
platelet count/spleen diameter ratio	431.6±116.1	1099.7±356.5	10.84	<0.001**

**Highly significant difference

Table (3): Diagnostic validity of blood ammonia level and platelet count/ spleen diameter ratio for detection of varices

	AUC	Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy
Ammonia (umol/ L)	0.991	74.0	97.5%	80%	90.7%	94.1%	91.7%
Platelet count/spleen diameter ratio	0.999	638.9	100%	97.5%	95.2%	100%	98.3%

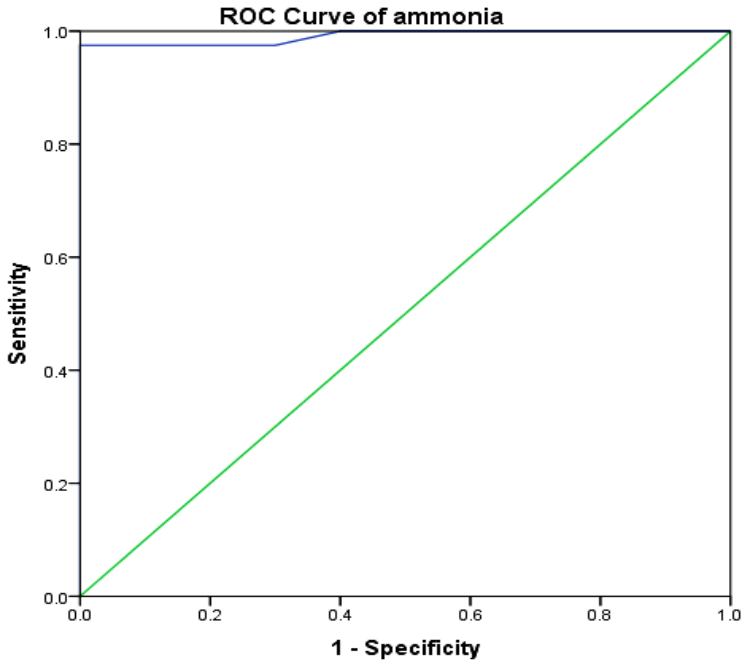

Fig. (1): ROC curve for the diagnostic validity of blood ammonia level in detection of oesophageal varices.

Table (4): Spearman correlation between size of varices and ammonia and platelet count spleen diameter ratio

	Size of varices	
	r	**P value**
Ammonia (umol/ L)	0.692	<0.001**
platelet count/ spleen diameter ratio	-0.461	0.003*

**Highly significant difference
* Significant difference

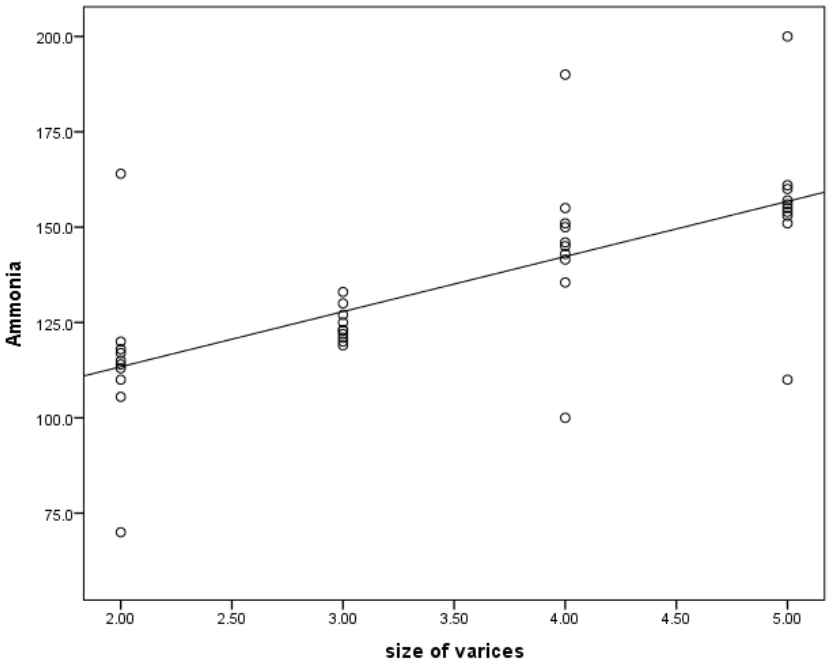

Fig. (2): Spearman correlation between blood ammonia concentrations and size of varices

Table (5): Comparison between different degrees of varices among cirrhotic group regarding platelet count /spleen diameter ratio and blood ammonia level

	Cirrhotic group with grade I varices (group IIa) (no=10)	Cirrhotic group with grade II varices (group IIb) (no=10)	Cirrhotic group with grade III varices (group IIc) (no=10)	Cirrhotic group with grade IV varices (group II d) (no=10)	Kruskal Wallis Test	P value	Post Hoc Test
	Mean±SD	Mean±SD	Mean±SD	Mean±SD			
platelet count /spleen diameter ratio	529.7±100.9	433.3±85.5	400.3±50.3	362.9±145.9	9.92	0.02*	P1=0.19 P2=0.02* P3=0.05* P4=0.89 P5=0.75 P6=0.98
Ammonia (umol/ L)	114.7±22.6	124.3±4.5	145.7±21.9	155.7±21.4	9.73	<0.001**	P1=0.27 P2=0.001* P3=<0.001** P4=0.02* P5=0.001* P6=0.25

*Significant difference **Highly Significant difference

1------ Comparison between groupIIa and groupIIb .
2------ Comparison between groupIIa and groupIIc.
3------ Comparison between groupIIa and groupIId .
4------ Comparison between groupIIb and groupIIc .
5------ Comparison between groupIIband groupIId .
6------ Comparison between groupIIc and groupIId

Table (6): Diagnostic validity of blood ammonia level and platelet count/spleen diameter ratio for detection of large varices [grade III and IV)

	AUC	Cutoff point	Sensitivity	Specificity	PPV	NPV	Accuracy
Ammonia	0.934	102	95%	52.5%	50%	95.5%	66.7%
Platelet count/ spleen diameter ratio	0.864	436.5	82.5%	70%	84.6%	66.7%	78.3%

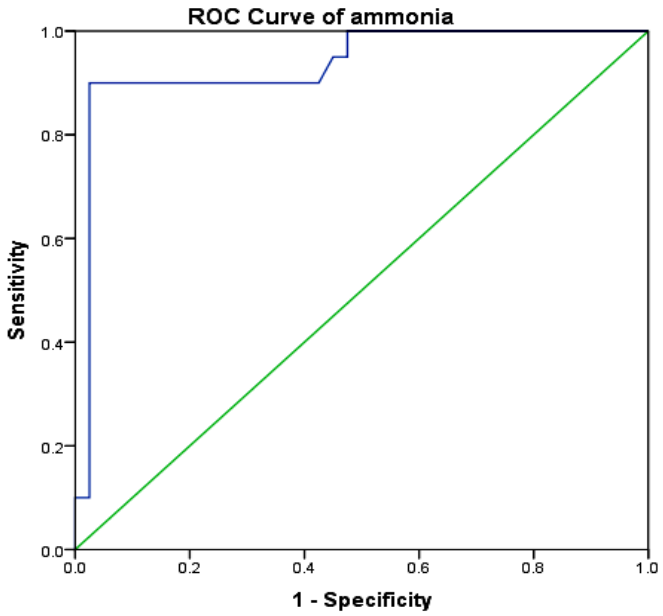

Fig. (3): ROC curve for the diagnostic validity of blood ammonia level in detection of large oesophageal varices

DISCUSSION

The portal venous system has numerous collaterals that interconnect with the systemic circulation. When portal pressure rises above 10 mmHg, potential portosystemic collaterals may develop. Formation of collaterals is a complex process involving the opening, dilatation and hypertrophy of pre-existing vascular channels. It is possible that active neoangiogenesis is involved in the formation of collateral vessels **[10]**.

Portosystemic shunts have been shown to be responsible for recurrent or persistent Porto-systemic encephalopathy **[11]**. Ammonia plays a major role in the pathogenesis of hepatic encephalopathy in cirrhotic patients **[12]**.

The generated ammonia, which reaches the liver through the portal vein, is converted to urea by means of urea cycle and excreted from the kidneys. In patients with decreased hepatic functional reserve or those with portosystemic shunt, ammonia level in the blood rises **[8]**.

This study was conducted to evaluate the role of blood ammonia as a non invasive marker for detection of oesophageal varices (O.V) and also for presence of large varices and compared it with platelet count/ spleen diameter ratio that is one of the most important non invasive predictors of O.V.

Blood ammonia values were estimated in cirrhotic groups and control group. The study showed that there was a highly significant difference between the mean ammonia level of cirrhotic and control groups (P<0.001). The mean values of serum ammonia in cirrhotic groups was 111.9±39.1 umol/L while it was 45.5±4.4 umol/L in control group. This was in agreement with the study done by Khondaker et al. **[9]** that found that there was a significant increase in blood ammonia level in patients with liver cirrhosis in comparison to healthy individuals but with a low

level of serum ammonia in healthy individuals, that was 28.47 umol/L.

On the other hand, there was a highly significant increase in the mean values of serum ammonia in cirrhotic patients with varices in comparison to other patients without varices. The best cutoff value for detection of O.V was 74 umol/L with sensitivity 97.5% and specificity 80%, that comes in agreement with the study done by El-Hefny et al. **[16]** with a cutoff value of 77.5 umol/L with sensitivity 100% and specificity 95% for detection of O.V, also with the study done by Terantino et al. **[8]** with different cutoff value that was 42 umol/L with sensitivity 97% and specificity 43% for detection of O.V.

This study found that, there was a highly significant decrease in the mean values of platelet count/ spleen diameter ratio in cirrhotic patients with varices in comparison to other patients without varices. The best cutoff value for detection of O.V was 638.9 with sensitivity 100%, specificity 97.5%, PPV 95.2% and NPV 100%, that comes in agreement with the study done by Baig et al**. [14]** with a cutoff value of 1014 with sensitivity 98.1% and specificity 88.6% for detection of O.V.

In the present study, there was a highly significant positive correlation between serum ammonia and size of varices, r (0.692) and P value (<0.001). This comes in agreement with study done by Khondaker et al. **[9]** that found that there was a significant correlation between blood ammonia level and size of varices, r (0.451), P value (<0.05).

On the other hand, there was a significant negative correlation between platelet count/spleen diameter ratio and size of varices, r (-0.461) and P value (<0.05). This comes in agreement with study done by Thomopoulos et al. **[18]** that reported that there was negative correlation between platelet count/ spleen diameter ratio and size of varices, r (-0.431) and P value (<0.05).

In the present study, there was a significant decrease in the mean values of platelet count/spleen diameter ratio in cirrhotic patients with evidence of grade III and IV varices (large varices) in comparison to cirrhotic patients with grade I and II varices. The best cutoff value for detection of large varices was 436.5 with sensitivity 82.5%, specificity 70%, PPV 84.6% and NPV 66.7%. This comes in agreement with study done by Sarangapani et al. **[15]** that found that a cutoff point of 909 had a sensitivity of 88.5% and specificity of 83.5% for detection of large varices.

Blood ammonia, the newly suggested non-invasive marker of esophageal varices showed significant increase in cirrhotic patients with endoscopic evidence of grade III and IV oesophageal varices (large varices) in comparison to cirrhotic patients with grade I and II varices (P<0.05) in the present study.

To test the blood ammonia level as a predictor of large varices, sensitivity and specificity of blood ammonia level at different cutoff values were assessed. Blood ammonia at 102 umol/L had sensitivity of 95% and specificity of 52.5% in detecting large oesophageal varices (grade III and IV varices) in patients with liver cirrhosis. Its PPV was 50% and NPV was 95.5% with accuracy of 66.7%. This comes in agreement with study done by Montasser et al **[17]** with a cutoff value of 133 umol/L with sensitivity 100% and specificity 96% in detecting large varices, also with study done by Khondaker et al. **[9]** that found a significant correlation between blood ammonia level and size of varices, but with a different cutoff point of about 63 umol/l with sensitivity of 95% and specificity of 50% in detecting large oesophageal varices in patients with cirrhosis.

This study proved that blood ammonia level may be a non invasive marker for presence of oesophageal varices and also for detection of large varices. A cutoff value of 74 umol/L for presence of varices and 102 umol/L for detection of large varices. The study compared between blood ammonia level and platelet count/ spleen diameter ratio, that was one of the most important non invasive marker for prediction of O.V, that found that, detection of oesophageal varices was more accurate with platelet count/spleen diameter ratio, but for detection of large esophageal varices, blood ammonia level was more accurate.

Strict precautions should be done for collection, handling, storage and analysis for blood samples to avoid errors for determination of ammonia, also diet and body mass index may affect blood ammonia level **[13]**, this explained different cutoff values of this study and other Egyptian studies from studies done in Italy by Terantino et al. **[8]** and in Bangladesh by Khondaker et al**. [9].**

CONCLUSION

So finally, it can be concluded that blood ammonia level can detect oesophageal varices at a cutoff value of 74 umol/L and can detect large varices at a cutoff value of 102 umol/L, so blood

ammonia level may be a good tool for non invasive prediction of oesophageal varices.

Funding: None.
Conflicts of interest: None.
Ethical approval: Approved.

REFERENCES

1. Poynard T, Bedossa T , Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR and DOSVIRC groups . *Lancet* 1987; 349 : 825 – 32 .
2. De franchis R . Revising consensus in portal hypertension : Report of the Baveno V consensus work shop on definitions, methodology and therapy in portal hypertension. *J Hepatol* 2010; 53:762-68.
3. Merli M, Nicolini G, Angeloni S, Rinaldi V, De Santis A, et al. Incidence and natural history of small esophageal varices in cirrhotic patients. *J Hepatol* 2003;38:266-72.
4. Augustin S, Millan L , Gonzalez A. Detection of early portal hypertension with routine data and liver stiffness with asymptomatic liver disease. A prospective study . *J.Hepatol*.2013 ;22:250-60.
5. Lubel JS , Angus PW. Modern management of portal hypertension .*Internal med.J*. 2005; 35[1): 45-49.
6. Garcia-Pegan , Garcia-sancho. Functional aspects on pathophysiology in portal hypertension in liver cirrhosis . *J Hepatol* 2012 ; 57[2),458-61.
7. Berzigotti A, Gilabert R , Abraldes JG. Non invasive prediction of clinically significant portal hypertension and esophageal varices in patients with compensated liver cirrhosis. *Am.J. Gastroenterol*. 2008 ; 103[5),1159-67.
8. Tarantino G, Citro V, Esposito P, Giaquinto S, de Leone A, et al . Blood ammonia levels in liver cirrhosis: A clue for the presence of portosystemic collateral veins. *BMC Gastroenterol* 2009;9:21.
9. Khondaker M, Ahmad N, Al-Mahtab M , Sumi S. Correlation between blood Ammonia Level and oesophageal Varices . *Euroasian J Hepato-Gastroenterol*. 2013; 3: 10-14.
10. Bosch J, Navasa M, Garcia-Pegan JC, Delacy AM , Rodes J. Portal Hypertension. *Medical Clinics of North America* 1989; 73:931-953.
11. Riggo O, Erafti C, Catalano C, Pedeconi F, Mecarelli O et al. High prevalence of Spontaneous Portal-Systemic Shunts in persistent hepatic encephalopathy: A case control study. *J Hepatol*.2005; 42: 1158-1165.
12. Butterworth RF, Giguere JF, Michaud J, Lavoie J Layrargues GP. Ammonia is a key factor in the pathogenesis of Hepatic Encephalopathy. *Molecular and chemical Neuropathology*, 1987; 6: 1-12.
13. Howanitz JH, Howanitz PJ , Skrodzi CA. Influences of specimen processing and storage condition on results for plasma ammonia. *Clin Chem*. 1984; 30:906-908.
14. Baig.WW, Nagaraja MV , Varma M. Spleen diameter for diagnosis of O.V.: Is it feasible? *J Gastroentrol*. 2008 ; 22: 852-8.
15. Sarangapani A, Shanmugam C, Kalyanasundaram M, Rangachari B, Thangavelu P , Subbarayan JK. Non invasive prediction of large O.V. in CLD. *Saudi Journal of Gastroentrology*. 2010; 16[1):38-42.
16. El-Hefny N, Hareedy M and Abdel-Aal R*:* Blood ammonia level and other non invasive parameters as indicators of portosystemic collaterals in cirrhotic patients: Thesis submitted for partial fulfillment of MSC degree in internal medicine . Assuit university 2013.
17. Montasser M, Abdella H , Samy A. Evaluation of venous ammonia level, splenic longitudinal diameter and portal vein diameter as non invasive indicator for portosystemic collaterals in Egyptian cirrhotic patients . *Open journal of Gastroenterology* 2014; (4) 265-74.
18. Thomopoulos KC, Labropoulou-Karatza C, Mimidis KP, Katsakoulis EC, Iconomou G , Nikolopoulou V.N. Non invasive predictors of the presence of large oesophageal varices in patients with liver cirrhosis. *Digestive and Liver disease* 2003;35 :473-478.

Peer reviewer: Tarik Zaher ,Professor of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt.
Editor: Mohamed Emara, Lecturer of Tropical Medicine and Hepatogastroenterology, Faculty of Medicine, Zagazig University, Egypt