Original Article

BLOOD AMMONIA LEVEL PREDICTS THE PRESENCES OF ESOPHAGEAL VARICES IN PATIENTS OF LIVER CIRRHOSIS DUE TO HEPATITIS B AND C VIRUSES

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Objective: To determine the biochemical and ultrasonographic non invasive parameters in liver cirrhotic patients due to Hepatitis B and C virus. To validate the non invasive parameters with the presence of esophageal varices using endoscopy as gold standard.

Material and Methods: Two hundred diagnosed patients were taken. Every patient underwent esophagogastroduodenoscopy (EGD) for the presence of esophageal varices. Ultrasonography was done to calculate the spleen diameter. Blood samples were taken to find the levels of blood ammonia and platelet count.

Results: On the basis of Receiver operating curve (ROC) blood ammonia level had area under the curve (AUC) 1.000 (p<0.001) platelet count/spleen diameter ratio (AUC=0.008, p<0.001) platelet count (AUC 0.009, p<0.001) and spleen diameter had area under the curve 0.986 (p<0.001). The maximum area under the curve was observed with blood ammonia (100%) as compared to other parameters.

Conclusion: It was concluded from the present study that blood ammonia level is the most reliable non invasive parameter in predicting the presence of esophageal varices as compared to other non invasive parameters.

Key words: Esophagogastroduodenoscopy, Receiver operating curve, Area under the curve.

Introduction

Liver cirrhosis is the chronic ongoing injury to the liver leading to extensive scaring and impairment in its function. Liver cirrhosis has a worldwide incidence of 5-10%.[1] The most common cause in our country is viral hepatitis as compared to alcohol in the west. Cirrhosis causes irreversible damage and is commonly accompanied by portal hypertension leading to development of esophageal varices.

Esophageal varices have a mortality rate of 17-57%.[2] According to recent recommendation, every diagnosed liver cirrhotic patient should undergo endoscopy for the presence of esophageal varices. Endoscopy is an invasive and expensive procedure; therefore the non invasive predictors of esophageal varices need to be determined. In liver cirrhosis, platelet count is low that is thrombocytopenia. There are number of factors causing thrombocytopenia. Portal hypertension is the major cause of thrombocytopenia. In enlarged spleen, the platelets are sequestrated thus reducing their mean life time leading to low palatelet count. There is reduction in production of thrombopoieten which is required for platelet formation. Thrombocytopenia is also due to myelotoxic effect of alcohol or hepatitis viruses[3]. Presence of antithrombocytic antibodies and thrombocytes associated immunoglobulins, which can be found in the sera of liver cirrhotic patients[4].

In patients with liver cirrhosis, portal hypertension leads to vascular disturbances causing congestion of the red pulp which ultimately causes splenomegaly. Normal size of spleen is 12cm pole to pole that is longitudinal diameter[5]. Ammonia is predominantly derived from protein degradation. Most of the ammonia in the blood comes from the intestine by the action of bacterial proteases, ureases and amine oxidases and also by deamination of glutamine in the small and large intestines Portal vein carries blood from intestines to liver that's why portal vein ammonia concentration is five to ten folds higher than in systemic circulation.

In the liver hepatocytes, 85% of ammonia is converted back into urea by urea cycle which is less toxic product. The kidney is the site for ammonia removal in the form of ammonium ions in the urine. Two factors contribute to hyperammonemia in liver cirrhosis;

1. In liver cirrhosis, there is decreased mass of functioning hepatocytes, resulting in fewer opportunities for ammonia to be detoxified in the liver.
2. There is portosystemic shunting which can divert ammonia containing blood away from the liver to the systemic circulation.

There are different controversies regarding the reliability of various non invasive parameters in predicting and grading esophageal varices. This study has provided information regarding the reliability of non invasive parameters in predicting and grading esophageal varices.

Materials and Methods

Informed consent was obtained from two hundred patients with hepatic cirrhosis after explaining the study protocol. Detailed history was taken and a clinical examination was performed according to inclusion and exclusion criteria.Ultrasonographic examination was performed to determine the spleen longitudinal diameter. Blood samples were taken to find the levels of platelet count and blood ammonia level. Every patient than undergo endoscopic examination and divided into two groups;

Group I: Patients having no esophageal varices.
Group II: Patients having esophageal varices.

The data was entered and analyzed using SPSS 17.0 (Statistical Package for Social Sciences) Mean $\pm$ SD were given for normally distributed quantitative variables. Frequencies and percentages were given for qualitative variables. Graphs were presented for both qualitative and quantitative variables. One-Way ANOVA was applied to compare the means difference of quantitative parameters between the groups and post-hoc Tukey's test was applied to observe which groups mean differed. ROC curves were plotted as sensitivity Vs 1-specificity. A p-value of <0.05 was considered as statistically significant.

Results

Gastroesophageal Varices:

The patients having no varices were 59 but 141 patients were having esophageal varices. **(Table.1)** Comparison of means between biochemical and other parameters.

Table-1: Baseline characteristics of the patients with liver cirrhosis.

Characteristics of Patients	Liver cirrhotic patients (n=200)
Esophageal varices	
No varicxes	59
Varices present	141

Blood ammonia:

The mean $\pm$ SD blood ammonia level (85.13$\pm$ 25.73 μM/L) was significantly higher (P=0.000) in patients with varices than the level (33.62 $\pm$ 2.63 μM/L) in patients without varices. (Table.2)Maximum and minimum values of blood ammonia level in patients having no vaiceswas 29-40 μM/land 41-140 μM/l in patients with having varices. **(Table.2) (Fig.1)**

Platelet count:

The mean$\pm$SD platelet count (58166.67$\pm$41541.21/ml) was significantly lower (P=0.000) in patients with varices than the level (229838.98$\pm$67845.03/ml) in patients without varices. **(Table-2)** Maximum and minimum values of platelet count in patients having no vaices was 110000-352000/ml and 4400-76000/ml in patients with having varices. **(Table-3) (Fig-2)**

Spleen diameter:

The mean$\pm$SD spleen diameter (15.4050$\pm$1.56cm) was significantly larger (P=0.000) in patients with varices than the value (12.7322$\pm$0.49cm) in patients without varices. **(Table-2)** Maximum and minimum values of spleen diameter in patients having no vaices was 12.0-13.5 cm and 12.5-19.5 cm in patients with having varices. **(Table.3) (Fig.3)**

Platelet count/ spleen diameter ratio:

The mean $\pm$ SD platelet count/ spleen diameter ratio in patients with no varices was 18087 $\pm$ 5435.35 and in patients having varices was 4020.19$\pm$ 3128.61.So, the platelet count/ spleen diameter ratio in patients with varices was significantly lower (p=0.000) than in patients without varices (Table.2)Maximum and minimum values of platelet count/spleen diameter ratio in patients having no vaices was 8222.2- 29090.9 and 25120-240.4 in patients with having varices. **(Table.3)(Fig.4)**

Receiver operating curve:

The receiver operating curve (ROC curve) for the four parameters was compared. Blood ammonia level had area under the curve 1.000(p=0.000 i.e. 100%)Platelet count/spleen diameter ratio (AUC=0.008, p=0.000 i.e. 8%), Platelet count (AUC 0.009, p=0.000 i.e. 9%) and spleen diameter had area under the curve 0.986 (p=0.000 i.e. 98%). The maximum area under the curve was observed with blood ammonia (100%) as compared to other parameters.**(Table.4)**

Table-4: Validation of non invasive parameters in predicting.

Parameters	Varices preesent (AUC)	P-value
Blood ammonia	1.0 (100%)	p=0.00*
Platelet count	0.009 (9%)	p=0.000*
Spleen diamter	0.986 (98%)	p=0.000*
Platelet count/spleen diameter ration	0.008 (8%)	p=0.000*

Table-2: Comparison of non invasive parameters among patients having no varices and varices.

Parameters	No Varices N=59 Mean±SD	Varices Present N=141 Mean±SD	P value
Blood ammonia μm/1	33.62±2.63	85.13±25.73	0.000*
Platelet count/ml	229838±67845.03	58166±41541.21	0.000*
Spleen diameter (cm)	12.73±0.49	15.40±1.56	0.000*
Platelet count/spleen diameter ratio	18087±5435.35	4020±3128.61	0.000*

P<0.05 is considered significant

Table-3: Maximum and minimum values of non invasive parameters in patients having no vaices and varices.

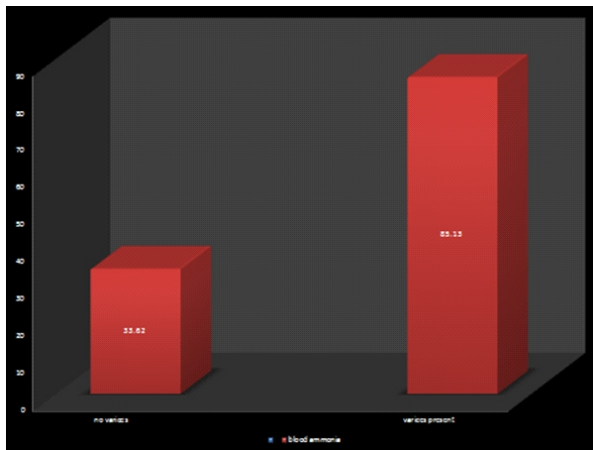| Blood ammonia μm/1	Platelet count/ml	Spleen diameter (cm)	Platelet count/spleen diameter ratio
No varices 29 - 40 μM/1	110000-352000/ml	12.0-13.5cm	8222.2-29090.9
Varices 41 - 140 μM/1	4400-76000	12.5-19.5cm	25120-240.4

Fig-1: Comparison in the levels of blood ammonia in patients with and without varices.
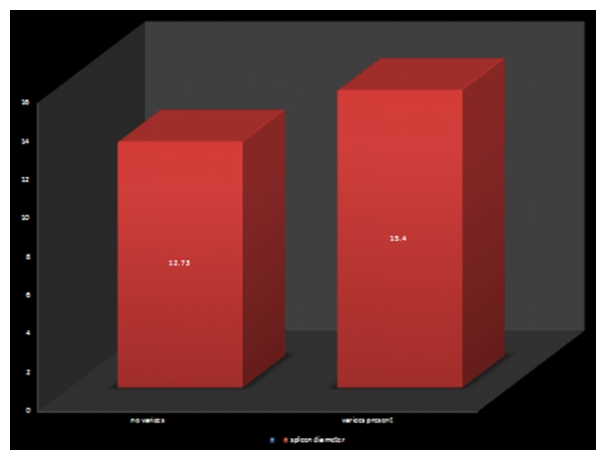

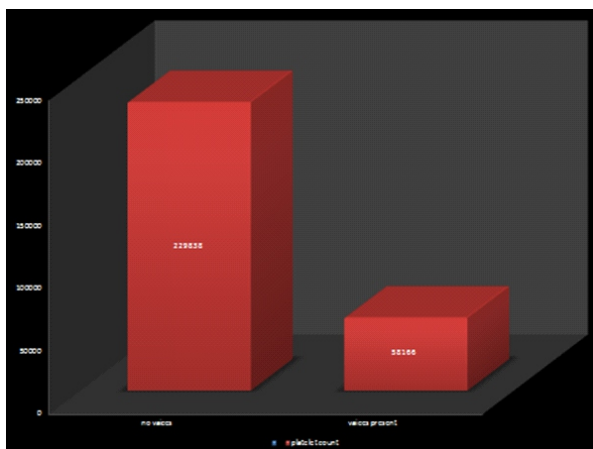**Fig-3:** Comparison in the levels of spleen diameter in patients with and without varices.

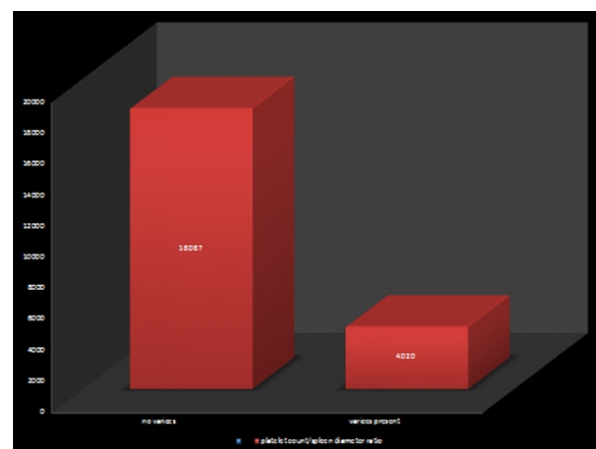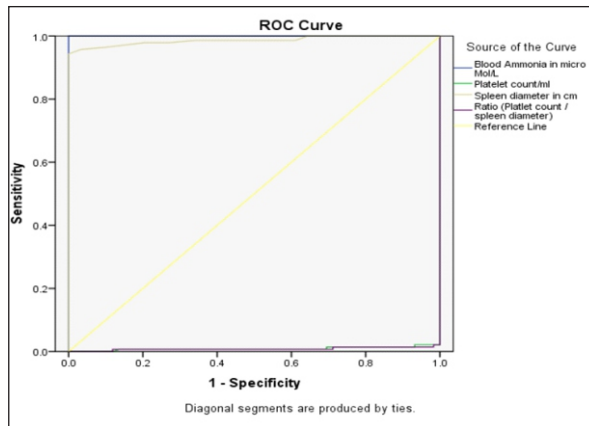**Fig-2:** Comparison in the levels of platelet count in patients with and without varices.

Fig-4: Comparison in the levels of platelet count/spleen diameter in patients with and without varices.

ROC Curve

Diagonal segments are produced by ties.

Discussion

Portal pressure increases in liver cirrhosis leading to development of varices and bleeding episodes, which may be massive and life threatening.[6]

In the present study, all those parameters which are affected by increase in portal pressure like blood ammonia, platelet count/spleen diameter ratio, platelet count and spleen diameter have been investigated. This study proved that blood ammonia level is the most sensitive and specific non invasive parameter in predicting the presence of esophageal varices in liver cirrhotic patients as compared to platelet count/spleen diameter ratio,platelet count and spleen diameter. Maximum area under the receiver operating curve i.e., 100% was observed for blood ammonia level.[7] Various studies proved that platelet count/spleen diameter ratio as the most reliable parameter in predicting presence of varices showing maximum area under the curve receiver operating curve 8-----15. The present study, didn't prove platelet count/spleen diameter ratio in detecting esophageal varices having AUROC=8%

Portal hypertension leads to splenomegaly, which is also considered to be a better predictor of esophageal varices.[16,17,18,19]The resultscame out from various studies that platelet count could be used as a predictor of esophageal varices.[20,21,22,23] Platelet count and spleen diameter are both highly interrelated because with increase spleen diameter there is greater sequestration of platelets. Also, in patients with alcoholic cirrhosis the platelet count decreases due to folate deficiency[24] so, the platelet count/spleen diameter is not concluded as a sensitive parameter in predicting presence and grading of esophageal varices. All the previous studies that predicted parameters other than blood ammonia didn't include hepatitis C patients.

The present study validated blood ammonia level as the most reliable parameter for predicting the presence of esophageal varices.The other reason of concluding blood ammonia as most reliable parameter may be that in the present study most of the patients were having hepatitis C as the cause of liver cirrhosis. In HCV patients, the portal pressures are relatively stable[24]. Blood ammonia is more related to damage in liver function as happened in progressive liver cirrhosis as compared to portal pressure so; it came out to be more reliable parameter. The source of ammonia in blood is gut, kidney and muscles. The ammonia is metabolized in the liver. As the liver dysfunction starts due to liver cirrhosis, there is development of portal hypertension and shunting of blood from the liver. As ammonia is not being metabolized, its level rises in the blood with the severity of liver damage and portal hypertension leading to development of esophageal varices. Ammonia can increase vascular tone of the vessels by causing influx of extracellular calcium through the voltage-dependent calcium channels and leading to worsen the portal hypertension.[25]

The present study evaluated that the patients with blood ammonia level below 40μM/l had very less chances of having varices. This study validates that blood ammonia level is the most sensitive and specific non invasive parameter in predicting presence of esophageal varices and also prediction of medium and large varices. This validation study does mean that we are recommending to replace endoscopy with blood ammonia level but this study is pointing that when is the need of endoscopy to avoid complications and unnecessary interventions.

Conclusion

The blood ammonia has advantage over other parameters as it is very much cost effective test as compared to other non invasive parameters in predicting esophageal varices. This non invasive parameter will help to predict presence of esophageal varices very cost effectively. The cost of an endoscopy in our country is Rs 2000 5000 while the cost of blood ammonia level is Rs 200-300. Thus Rs 1800 4800 is saved when an unnecessary endoscopy is avoided. This will ease the medical, social and economic burden of the patients. It is validated from the present study that blood ammonia level is the most reliable non invasive parameter as compared to platelet count/spleen diameter ratio, platelet count and spleen diameter in predicting presence of esophageal varices.

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