

Measuring Partial Pressure of Ammonia in Arterial or Venous Blood VS total Ammonia Levels in Hepatic Encephalopathy

Mohammad Asif Mehmood, Tariq Waseem, Farina Zia Ahmad, Malik Asif Humayun

Mohammad Asif Mehmood, Department of Medicine, King Edward Medical University/Mayo Hospital Lahore, 54000, Pakistan
Tariq Waseem, Malik Asif Humayun, Medical Unit I, Allama Iqbal Medical College/Jinnah Hospital Lahore, 54550, Pakistan
Farina Zia Ahmad, Department of Medicine, DHQ Hospital Faisalabad, 38000, Pakistan

Correspondence to: Malik Asif Humayun, Medical Unit I, Allama Iqbal Medical College/Jinnah Hospital Lahore. 54550, Pakistan. doctormalikasif@gmail.com

Telephone: +923336103130

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ABSTRACT

AIM: To find out the correlation between clinical grade of hepatic encephalopathy and different serum ammonia levels, namely, serum arterial ammonia, serum venous ammonia, partial pressure of ammonia in arterial plasma and partial pressure of ammonia in venous plasma.

METHODS: After informed consent from patients or their relatives, 100 patients in hepatic encephalopathy due to advanced liver disease, were examined for their clinical grade of hepatic encephalopathy within 24 hours of admission. Fasting arterial and venous blood samples were collected and sent to lab within 30 min. Blood pH was measured in both samples for each patient. Ammonia levels were measured directly by an enzymatic UV-Method. Partial pressure in a given sample was calculated by the recommended formula, using total ammonia and blood pH of the respective sample.

RESULTS: Maximum number of patients (53%) in this study was in clinical grade I of hepatic encephalopathy, 30% were in grade II, and 12% were in Grade III. Only 5% were in grade IV. While comparing means of serum arterial ammonia (0.26049), serum venous ammonia (0.264), partial pressure of ammonia in arterial blood (4.520) and partial pressure of ammonia (4.357) in venous blood with clinical grades of hepatic encephalopathy, they increased with increasing clinical grade of hepatic encephalopathy with *p*-values of 0.001, 0.01, 0.002, and 0.003 respectively. Partial pressure of ammonia in venous blood was most strongly associated with hepatic encephalopathy (*p*-value 0.001) followed by serum venous ammonia (*p*-value 0.002).

CONCLUSIONS: Total arterial ammonia, total venous ammonia, partial pressure of arterial ammonia, and partial pressure of venous

ammonia, all are positively and significantly correlated with worsening clinical grades of hepatic encephalopathy. Partial pressure of ammonia in venous blood followed by that in serum venous ammonia were most strongly associated with grade of hepatic encephalopathy.

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Key words: Hepatic encephalopathy; Ammonia; Partial pressure of ammonia; Liver failure

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INTRODUCTION

Hepatic encephalopathy is a serious neuropsychiatric syndrome of liver disease. Its manifestations range from mild cognitive dysfunctions to deep coma. Ammonia is believed to be the most important and primary causative factor of hepatic encephalopathy, and the work by Lockwood *et al*^[1] and Clemmesen *et al*^[2] further supported this hypothesis. However, correlation between ammonia levels and grades of hepatic encephalopathy remains controversial^[3]. Some studies document that clinical grades of hepatic encephalopathy are correlated with total ammonia concentrations in blood indicating towards brain exposure to total ammonia^[4]. Contrary to the usual clinical practice of assessing arterial ammonia concentration in hepatic encephalopathy, Kramer *et al*^[5] suggested the use of partial pressure of arterial ammonia as a better diagnostic tool in hepatic encephalopathy. In 2000, Kramer *et al*^[5] did a study to find out correlation between partial pressure of ammonia and clinical grades of hepatic encephalopathy and found that partial pressures of ammonia are strongly associated with clinical grades of hepatic encephalopathy. Later, Ong *et al* in 2003 disproved this hypothesis with a relatively larger study, when they concluded that there is no better correlation of partial pressure of ammonia with hepatic encephalopathy grades as compared to venous ammonia or arterial ammonia concentration^[6]. In 2003, Nicolao F *et al* came up with the same result that despite the significant correlation between partial pressure of ammonia and hepatic encephalopathy, neither partial pressure of ammonia nor arterial ammonia are, from a clinical point

of view, more useful than venous ammonia levels^[7]. Fewer studies have focused correlation of ammonia or partial pressures of ammonia with hepatic encephalopathy. This study was planned to resolve the apparent inconsistencies that were found in previous studies and to find out definitive benefit of measuring partial pressure of ammonia in arterial or venous blood over total arterial or venous ammonia levels in patients of hepatic encephalopathy in our population. This would guide about the most suitable test that may help the clinicians establish the diagnosis of hepatic encephalopathy with better supportive laboratory evidence.

MATERIALS

It was a cross sectional, analytical study carried out in the medical department of Jinnah Hospital, Lahore from 6th June 2009 to 31st June 2010.

All the patients with liver cirrhosis and hepatic encephalopathy of <48 hour duration were included in this study. Liver cirrhosis was defined by prior biopsy or typical clinical features of decompensated liver cirrhosis coupled with ultrasonographic findings. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethical Committee of Allama Iqbal Medical College. The need for written informed consent was waived in patients who were not able to consent. No restrictions were made as to the age, sex, and grades of cirrhosis when patients were selected. Exclusion criteria were: previous hepatic encephalopathy, hepatocellular carcinoma, renal failure (creatinine >2 mg/dL), hyponatremia (Na <130 mEq/L) at the time of the study, Wilson's disease, alcohol addiction before the enrolment, uncontrolled diabetes (blood sugar >300 mg/dL or <100 mg/dL), encephalopathy with or due to other organ failure like respiratory failure, heart failure, These patients were stratified into four groups according to clinical grade of hepatic encephalopathy.

Apart from complete history and physical examination, all baseline investigations like Hemoglobin, Total leukocyte Count (TLC), Differential Leukocyte Count (DLC), Serum (S)/Na⁺, S/K⁺, Prothrombin time (PT), Activated partial thromboplastin time (APTT), S/albumin, Liver function test (LFTs), Blood (B)/urea, serum creatinine, blood sugar, abdominal ultrasonography, and arterial blood gases were carried out in all subjects.

Selected patients were evaluated for clinical grade of hepatic encephalopathy according to West Heaven criteria within 24 hours of admission in ward. Arterial and venous blood samples of these patients to measure ammonia levels were collected in heparinized vacutainer tubes. Samples were immediately centrifuged and sent to lab on ice pack within 30 minutes of collection. Total ammonia was measured directly by enzymatic UV-method with reagent obtained from Randox diagnostics (UK) in department of Pathology, Jinnah Hospital Lahore. Partial pressure of ammonia (gaseous non-ionized form of ammonia) was measured by following formula after getting blood pH in both arterial and venous samples.

$$p\text{NH}_3 = \frac{\text{Total ammonia nitrogen} \times 22.1}{\frac{[\text{H}^+] + 1 \times}{K^*a}}$$

Where X = 0.9 and K*a = 9.8 x 10

Ammonia measurements in four different forms were compared to determine whether partial pressure of ammonia serves as a better indicator of severity of hepatic encephalopathy than total ammonia levels in arterial and venous blood.

Data Analysis

All collected information entered into the computer and analyzed

using SPSS 17.0 soft ware. Data was analyzed descriptively and analytically. In descriptive section means and standard deviations were computed for quantitative variables while count and percentages were calculated for categorical variables. In analytical section, Analysis of variance (ANOVA) technique was used to compare the means of arterial, venous, partial pressure of arterial and partial pressure of venous ammonia levels with their four clinical grades. A Pearson correlation was applied to find out the strongest association factor with clinical grades of hepatic encephalopathy. *P*-value less than 0.05 was considered as statistically significant. Graphical comparison was also shown by line chart to see the associations of different ammonia levels with the clinical grades.

RESULTS

Total 100 cirrhotic patients, irrespective of etiology, fulfilling inclusion and exclusion criteria were enrolled in this study. Out of these, 66 were males and 34 were females. Base line characteristics of study population are described in table 1. These characteristics include age, gender, etiology, stage of liver disease, clinical grades of encephalopathy, precipitating factor for hepatic encephalopathy, renal function and hemodynamic parameters. Most common etiological factor was Hepatitis C infection (75%). Upper GI bleeding (67%) was the most common precipitating factor for hepatic encephalopathy in our study population. Maximum number of patients (53%) was in grade I, 30% in grade II, 12% in Grade III, while 5% of the patients were in grade IV hepatic encephalopathy. While comparing means of serum arterial ammonia, serum venous ammonia, partial pressure of ammonia in arterial blood and partial pressure of ammonia in venous blood (Table 3 and 4), these increased with worsening clinical grade of hepatic encephalopathy with *p*-values of 0.001, 0.01, 0.002, and 0.003 respectively. While finding the correlation among all these (Figures 1 and 2), partial pressure of ammonia in venous blood was found to be most strongly associated with hepatic encephalopathy (*p*-value 0.000) followed by serum venous ammonia (*p*-value 0.002).

DISCUSSION

Our study revealed two important aspects; the first one being that ammonia levels in different blood samples, namely, total ammonia

Table 1 frequency distribution of clinical grades.

Variable	
Age (Years)	54.15±12.34
Male	66
Female	34
Etiology	
Hepatitis B	9
Hepatitis C	75
Others	16
Child's grade	
A	0
B	64
C	36
Child's score	8.63±1.42
Grade of Encephalopathy	
1	53
2	30
3	12
4	5
Blood Pressure (mmHg)	
Systolic	110. 83±12.67
Diastolic	72.33±7.95
ALT	75.03±61.19
Serum Albumin (mg/dL)	3.85±0.96
Serum Creatinine ¹ mg/dL	1.07±0.17

¹ Numerical Variables compared by *t*-test; ² Categorical variables compared by chi-square test.

Table 2 Precipitating factors of hepatic encephalopathy.

Constipation		Frequency	Percent
Constipation	No	59	59
	Yes	41	41
Hypovolemia	No	93	93
	Yes	7	7
GI bleed	No	33	33
	Yes	67	67
Infections	No	75	75
	Yes	25	25
Hypokalemia	No	78	78
	Yes	22	22

Table 3 Ammonia levels and partial pressure of ammonia in different samples in study patients.

Variable	Minimum	Maximum	Mean	Standard Error of Mean	Std. Deviation
Arterial ammonia (ug/dL)	0.062	0.99	0.26049	0.021	0.214
Venous ammonia (ug/dL)	0.09	0.96	0.264	0.023	0.233
Arterial partial pressure (mmHg)	1.008	17.263	4.520	0.377	3.778
Venous partial pressure (mmHg)	1.230	16.74	4.357	0.392	3.929

Table 4 Means and standard deviation of different ammonia levels in four clinical grades of hepatic encephalopathy.

Grade/Arterial ammonia	Arterial ammonia	Venous ammonia	Partial pressure arterial ammonia	Partial pressure venous ammonia
GRADE I ($n=53$)	0.22±0.22	0.22±0.23	3.97±3.95	3.52±3.69
GRADE II ($n=30$)	0.289±0.20	0.25±0.14	4.76±3.57	4.08±2.43
GRADE III ($n=12$)	0.23±0.09	0.42±0.35	3.86±1.40	7.07±6.20
GRADE IV ($n=5$)	0.60±0.04	0.47±0.06	10.47±1.58	8.30±2.12

Table 5a One way anova for arterial partial pressure.

		Sum of Squares	df	Mean Square	F	Sig.
Arterial ammonia	Between Groups	0.689	3	0.230	5.709	0.001
	Within Groups	3.862	96	0.040		
	Total	4.551	99			
Venous ammonia	Between Groups	0.597	3	0.199	3.961	0.010
	Within Groups	4.822	96	0.050		
	Total	5.418	99			

Table 5b One-way anova for venous partial pressure.

		Sum of Squares	df	Mean Square	F	Sig.
Arterial partial pressure	Between Groups	199.745	3	66.582	5.267	0.002
	Within Groups	1213.489	96	12.641		
	Total	1413.234	99			
Venous partial pressure	Between Groups	204.726	3	68.242	4.947	0.003
	Within Groups	1324.228	96	13.794		
	Total	1528.954	99			

levels in arterial and venous blood, and partial pressure of ammonia in arterial and venous blood, are positively correlated with worsening clinical grades of hepatic encephalopathy (Table 3 and 4). The second aspect it made clear was that partial pressure of ammonia in venous blood is relatively more strongly associated with worsening grades of hepatic encephalopathy among all four parameters of measurements (Figures 1 and 2).

The first inference drawn here in study is very much in coincidence with the available pool of data, which favors ammonia being the main culprit molecule in causation of hepatic encephalopathy^[8].

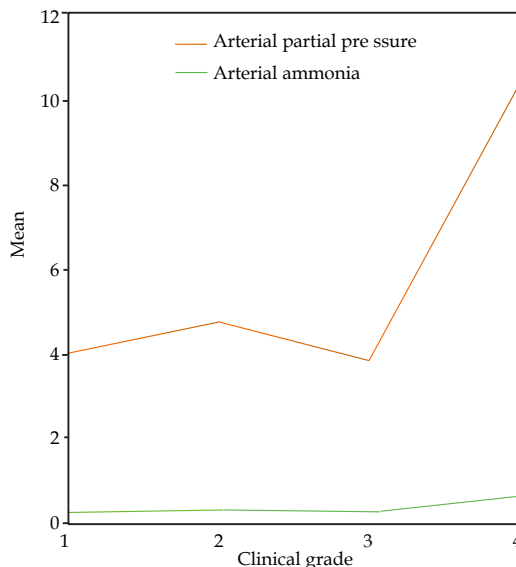


Figure 1 Graphical comparison of partial pressure & total arterial ammonia with their clinical grades.

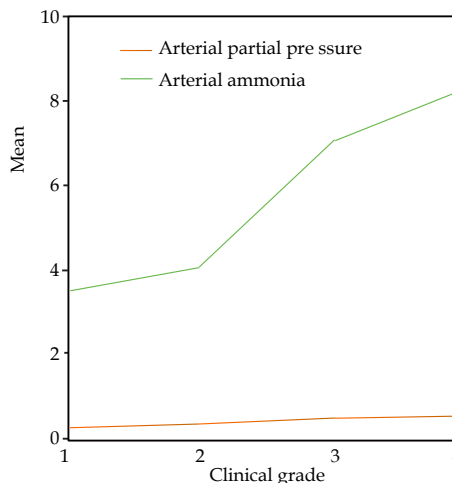


Figure 2 Graphical comparison of partial pressure & total venous ammonia with their clinical grades.

Increasing ammonia levels increase the exposure of brain to ammonia and the risk of ammonia-induced neuronal injury through various mechanisms is exaggerated by this exposure. Whether this hyperammonemia is measured in lab as total ammonia levels or partial pressure of ammonia, a positive correlation of all these with clinical manifestations established that ammonia was the main pathogenic factor in appearance of hepatic encephalopathy in our study.

The second inference drawn from this study bears some important outcomes. Our study showed that partial pressure of ammonia in venous blood is more closely associated with clinical manifestations of hepatic encephalopathy (Table 4). Kramer *et al* in 2000 pointed out that non-ionized free available ammonia in blood might have a better permeability through blood-brain barrier than total ammonia levels. Their observations showed that partial pressure of ammonia in arterial blood is more strongly associated with worsening clinical grades of hepatic encephalopathy than total ammonia levels. Unfortunately, they did not measure the venous total ammonia or partial pressure of ammonia in venous blood, which could broaden the results and impact of their study. Our study carries this advantage of measurements of ammonia in venous blood also. This definitely

raises a question that while arterial ammonia is a better measure of ammonia exposure to brain, how venous ammonia correlates better with the clinical situations. There is not enough data in literature to answer this query, but two reasons may help solve the situation. Firstly, the subjects with hepatic encephalopathy whenever studied have been so diverse with their clinical scenario that it is difficult to get a homogenous population in one study. Second, ammonia is a single element studied in pathogenesis of hepatic encephalopathy, while others elements may also be playing their role simultaneously, which act as confounding factors in study. In all studies comparing different ammonia levels in patients with hepatic encephalopathy^[3-5], there has been high degree of overlap in ammonia levels in different clinical grades of hepatic encephalopathy. It was the case in our study (Figure 1, 2), which might have precluded in defining the exact role of ammonia in pathogenesis of hepatic encephalopathy. This inference of our study is, however, in coincidence with study by Ong *et al* in 2003, who concluded that all four levels of ammonia (arterial ammonia, venous ammonia, partial pressure of arterial ammonia and partial pressure of venous ammonia) had a positive correlation with severity of hepatic encephalopathy. Although venous ammonia was shown better correlated with severity of hepatic encephalopathy, none was superior to the other in this regard. Our study also concluded almost the same way. Although we don't conclude that none has benefit over the other, we, however, may state that all four parameters of measurement have the same significant correlation with severity of hepatic encephalopathy. An alkaline pH is said to favor the free form of ammonia.

Recently, Senzolo *et al* in 2005 studied cirrhotic patients with minimal hepatic encephalopathy with their neuropsychological tests, EEG, arterial ammonia and partial pressure of ammonia in arterial blood. Arterial ammonia and partial pressure of ammonia were significantly correlated with abnormal EEG patterns but not with neuropsychological performance^[10]. In 2003, Nicolao *F et al*^[11] studied ammonia levels in arterial and venous blood and partial pressure of ammonia in arterial blood. They also concluded that although partial pressure of ammonia in arterial blood is more strongly associated with severity of hepatic encephalopathy, it couldn't be declared superior in routine clinical practice because the other ammonia levels also had positive correlation with severity of the clinical situation. Interestingly enough, they also did not measure partial pressure of ammonia in venous blood, so their result can't be well compared with our study in which we concluded partial pressure of ammonia in venous blood more useful among the four measurements. If we keep the results of Ong *et al*^[9] in mind, the only group of investigators who considered venous partial pressure of ammonia in their study and concluded that venous total ammonia levels followed by partial pressure of ammonia in venous blood are better correlated with the severity of hepatic encephalopathy, results of our study appear relevant. Our study further pointed out serum total venous ammonia has strongest positive correlation after partial pressure of venous ammonia. This is also in accordance with results by Ong *et al*. The study by Ong *et al* also included 25% patients with grade 0 hepatic encephalopathy while our study did not include any patient less than grade I hepatic encephalopathy. Moreover, most common precipitating factor of hepatic encephalopathy in study by Ong *et al* was azotemia. We intentionally excluded patients with renal insufficiency, which might have confounded the cause of altered conscious level. This also precluded patients with azotemia as main precipitant of their hepatic encephalopathy, to participate in our study.

An important shortcoming of our study was small number of patients in grade IV encephalopathy, the most severe form of

encephalopathy. This paucity of patients in this sub-group makes it difficult to generalize the results of this study uniformly to all patients with hepatic encephalopathy. The reason for this gross non-equality of patients in different clinical grades of hepatic encephalopathy was mainly our exclusion criteria. We excluded patients with concomitant other organ insufficiency which could affect our clinical diagnosis of encephalopathy due to hepatic cause only, or it could affect ammonia metabolism in some ways or the other. These concomitant organ failures commonly included renal insufficiency and respiratory failure, which could affect conscious level by virtue of their primary pathology or they could alter ammonia levels by alteration in acid base milieu of blood.

On the basis of results from our cross sectional study, we suggest that partial pressure of ammonia in venous blood should be considered in clinical practice for a better diagnosis. Since ammonia partial pressures correlate positively with clinical grades of hepatic encephalopathy, they can probably also be used for monitoring the response of treatment in hepatic encephalopathy. This, however, needs further studies before it can be made a part of the practice. Moreover, serum total venous ammonia levels were next to partial pressure of ammonia in venous blood in its association with severity of hepatic encephalopathy, they could also be used in clinical practice instead of arterial ammonia. This consequence of the study could have a pleasant outcome in clinical practice because patients with liver failure usually have bleeding diathesis, which makes arterial puncture uncomfortable for the patient as well as for the treating physician. We, however, suggest a large-scale trial including patients with more diverse etiological and pathophysiological nature of encephalopathy as well as measuring different ammonia levels with response to therapy. This could confirm the correlations not only in cross sectional way but also in a prospective method, making the practical implications more feasible in day-to-day clinical practice.

CONCLUSION

Total arterial ammonia, total venous ammonia, partial pressure of arterial ammonia, partial pressure of venous ammonia, all are positively and significantly correlated with worsening clinical grades of hepatic encephalopathy. Partial pressure of ammonia in venous blood followed by that in serum venous ammonia was more strongly associated with grade of hepatic encephalopathy.

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Peer reviewer: Sharon DeMorrow, PhD, Assistant Professor, Digestive Disease Research Center, Department of Internal Medicine, Texas A&M Health Science Center, College of Medicine, Scott & White Hospital, Olin E Teague Medical Center, 1901 South 1st Street, Building 205, Temple, Texas, 76504, USA.