



Homeostatic Model Assessment for Hyper Ammonemia-Harmonization Between Krebs TCA Cycle and Krebs-Henselit Urea Cycle Affected Ammonia Clearance in Liver Disease and Renal Failure

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ABSTRACT

Ammonia (NH₃) levels fluctuate widely in patients with hepatic encephalopathy, and excess NH₃ in brain leads to excessive accumulation of glutamine, which compromises astrocyte morphology and function accounting for the neurotoxicity induced complications, including altered consciousness and coma. Impaired renal function also causes both uremia and hyperammonemia, which seems to be the key substrate to diagnose encephalopathy. The present study estimates levels of blood NH₃ in both hepatic and uremic encephalopathy, and whether any correlation exists with other parameters of encephalopathy.

Cirrhotic Patients with Hepatic encephalopathy and Uremic encephalopathy were included in the study to estimate NH₃, liver and renal function and serum electrolytes.

NH₃ showed significant difference between both patients and control groups. All other parameters showed significant variations in levels between cases and controls; except for Serum Urea and Calcium, which did not alter significantly in hepatic encephalopathy, and anions levels, which showed no significant changes in uremic encephalopathy.

KEYWORDS : Hyper Ammonemia, Electrolytes, Encephalopathy

Introduction:

Ammonia (NH₃) has long been known for its poisoning effect on human blood. NH₃ levels fluctuate widely in patients with hepatic encephalopathy, and it is generally accepted that excess NH₃ in the brain leads to excessive accumulation of glutamine, which compromises astrocyte morphology and function. The osmotic effects of excess glutamine may account for the neurotoxicity of excess NH₃ at least in part, and it has been postulated that a brain glutamine synthetase inhibitor may be of therapeutic benefit, especially for the acute form of liver disease¹. NH₃ is also implicated in hepatic encephalopathy, where by its toxicity is mediated by glutamine, which is metabolized from NH₃ in the brain². NH₃ widens junctions in the blood-brain barrier, allowing not only pathogens, but also small molecules like glutamate along with neutrophils and water to penetrate the barrier³. Hyperammonemia may also result in irreversible brain damage⁴. Though NH₃ is toxic, systemic blood NH₃ levels are normal or only minimally elevated in uremia. Some of the symptoms of uremia especially nausea, vomiting, malaise and possibly bleeding are partly due to its intoxication with urea or a product of urea metabolism which could be NH₃⁵. In patients with severe liver diseases, impaired renal NH₃ excretion capacity may be a critical factor affecting plasma NH₃. There is a general consensus that gut-derived nitrogenous substances evade hepatic detoxification and affect central nervous system function⁶.

NH₃ exists in two forms in blood: ammonia ion (NH₄⁺) and unionized ammonia (NH₃). At physiological pH value the pKa for NH₃. Biological membranes are much more permeable to NH₃ than NH₄⁺, so only NH₃ freely passes through the blood-brain barrier. When blood pH increases, the ratio of NH₃/NH₄⁺ also increases, and the elevated NH₃ in the brain thus contributes to the cerebral dysfunction associated with hepatic failure⁶.

The acquired causes of hyper NH₃ are advanced in liver disease and renal failure. Encephalopathy, is usually taken place both in liver and renal failure, due to hyper NH₃, a highly toxic substances along with glutamate, neutrophils and water crossing the blood brain barrier into CNS leading to wide spectrum of Neuro-Psychiatric complications

including altered consciousness ultimately leading to coma and irreversible brain damage. Hepatic encephalopathy may occur in hepatitis or Cirrhotic patients, can be precipitated by GI bleeding, which enhances production of NH₃ in the colon leading to hyper NH₃. Impaired renal function also causes both uremia and hyperNH₃ having clinical symptoms of nausea, vomiting, malaise, electrolyte imbalance and Acid-Base imbalance.. Hyper NH₃, irrespective of either liver or renal diseases, is the key substrate leading to excessive accumulation of Glutamine (possibly antagonistic nature of both either over activation of the enzyme Glutamate synthetase, which incorporate NH₃ into Glutamate or inhibition of peripheral Glutaminase, actively involved in NH₃ Clearance) in the brain causes Cerebral dysfunction associated with other biochemical abnormalities like Electrolytes and acid-base imbalance. The rate of NH₃ entry into the brain is highly dependent on its plasma concentration and pH.

It has been postulated that a brain glutamine synthetase inhibitor may be of therapeutic benefit¹.

The present study was done to find out the significance of the levels of blood NH₃ in both hepatic and uremic encephalopathy, and whether any correlation exists with other parameters of encephalopathy.

Materials and Methods

38 Cirrhotic Patients with Hepatic encephalopathy and 48 Cirrhotic Patients with Uremic encephalopathy were included in the study. The diagnosis of hepatic encephalopathy was made after confirming the diagnosis of cirrhosis based on clinical and laboratory data. Also, patients were clinically diagnosed to have uremic encephalopathy. Control participants were recruited from the area where the patients are located using a systematic random sampling method. All control participants were medically healthy. We recruited 44 male and 42 female as a control subjects.

The study protocol was approved by the institutional Ethics Committee, and a written informed consent was obtained from all participants after signing before data collection.

For all patients initial samples were obtained immediately prior to starting treatment and after an overnight fast.

Fasting venous blood samples were collected through antecubital vein from the patients and control groups. The samples were analyzed to estimate NH₃, total protein, albumin, total bilirubin, direct bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, urea, creatinine, calcium and phosphorus, with commercially available assay kits (Meril Diagnostics) on a AutoQuant 400 auto analyzer and sodium, potassium, chloride and bicarbonate by Merilyte Electrolyte Analyzer.

Estimation of Ammonia: Biological Reference interval of arterial blood ammonia is 22-61 µg/dl. Several recent studies have suggested that it is not necessary to utilize arterial blood when measuring NH₃ in blood. Venous blood computation of the partial pressure of ammonia in blood sample may suffice. All the older methods have the interference values based on the arterial samples. Hence arterial blood reference values are given according the used methodology⁷.

Only recently venous samples have been used instead of arterial samples because of ease of collection less trauma and other advantages.

Demographic data was used to adjust for potential confounding factors or effect modifiers.

Appropriate quality assurance procedures and precautions are carried out to ensure reliability of the results. Also, both external and internal quality control was maintained, using quality control materials if Bio Rad, USA.

All statistical analysis were conducted using SPSS 22.0 statistical software package (SPSS, Chicago II,USA) for these analysis and Microsoft Office 2010, with statistical significance defined as an alpha level of 0.05.

Two tailed probabilities were applied throughout.

Independent 't'-tests were performed to compare the mean values of

continuous variables between the groups.

A logic regression analysis was performed to explore the association between blood biochemical variables (NH₃) levels and behavioral problems. In this model, the blood biochemical variables, followed by adjustment for age, education, material status, BMI, smoking and drinking.

Results :

The characteristics of the patients are shown in Table 1.

The demographic variables did not differ significantly between the patients and control groups.

Table 1: Demographic summary of the participants.

Parameters	Cirrhotic Patients with Hepatic encephalopathy (n = 38)	Cirrhotic Patients with Uremic encephalopathy (n= 43)	Control Groups (n = 86)
Age Years ± SD	55.6 ± 12.6	54.8 ± 8.2	54.5 ± 11.8
Sex (male/Female)	21 / 17	28 / 15	52 / 34
Smokers	58 %	68%	62%
Drinkers	73%	82%	68%
BMI	22.8 ± 2.6	23.2 ± 1.8	24.6 ± 3.2

Values Mean ± SD

The results of the various biochemical parameters subjects are tabulated in table 2. The various biochemical parameters were compared using students 't' test with significance of biochemical parameters between the control and diseased groups

Table 2: Liver and renal function indices among cirrhotic patients with hepatic encephalopathy, uremic encephalopathy and control groups

Parameters	Cirrhotic Patients with Hepatic encephalopathy (n = 38)	Cirrhotic Patients with Uremic encephalopathy (n = 43)	Control Groups (n = 86)	p Value: Cirrhotic Patients with Hepatic encephalopathy vs Control	p Value: Cirrhotic Patients with Uremic encephalopathy vs Control
Total bilirubin (mg/dl)	3.62 ± 1.21	1.23 ± 0.34	0.9 2 ± 0.31	<0.0001	<0.0001
Direct bilirubin (mg/dl)	1.29 ± 0.92	0.51 ± 0.32	0.33 ± 0.22	<0.0001	<0.0001
Alanine aminotransferase (U/L)	62 ± 12	32 ± 8	18 ± 4	<0.0001	<0.0001
Aspartate aminotransferase (U/L)	52 ± 11	26 ± 6	15 ± 3	<0.0001	<0.0001
Total Protein (gm/dl)	5.7 ± 1.52	5.9 ± 1.4	7.0 ± 0.8	<0.0001	<0.0001
Albumin (gm/dl)	2.2 ± 1.2	3.1 ± 1.2	4.0 ± 0.7	<0.0001	<0.0001
Alkaline phosphatase (U/L)	112 ± 26	118 ± 20	110 ± 28	0.7086	0.0972
Gamma Glutamyltransferase (U/L)	90 ± 29	46 ± 12	23 ± 18	<0.0001	<0.0001
Creatinine (mg/dl)	1.5 ± 0.4	8.6 ± 1.4	0.89 ± 0.3	<0.0001	<0.0001
Urea (mg/dl)	16 ± 8	145 ± 22	15 ± 4	0.4667	<0.0001
Calcium (mg/dl)	9.2 ± 2.2	9.0 ± 0.8	9.7 ± 0.9	0.0742	<0.0001
Phosphorus (mg/dl)	4.3 ± 0.8	5.7 ± 0.8	4.6 ± 0.5	0.0124	<0.0001
Sodium (mmol/L)	127 ± 3.3	135 ± 2.5	136 ± 2.0	<0.0001	0.0153
Potassium (mmol/L)	3.4 ± 0.8	4.9 ± 1.1	4.0 ± 0.5	<0.0001	<0.0001
Chloride(mmol/L)	78.0 ± 3.1	95.0 ± 2.7	96.0 ± 3.0	<0.0001	0.0676
Bicarbonate-Total CO ₂ (mmol/L)	31.8 ± 3.1	24.5 ± 2.6	24.3 ± 2.8	<0.0001	0.6961
Ammonia, NH ₃ (µg/dl)	142.6 ± 12.5	72.4 ± 11.6	37.8 ± 4.2	<0.0001	<0.0001

Values Mean ± SD; Statistical significance p < 0.05

The levels of total bilirubin, direct bilirubin, total protein, urea and creatinine were higher in patients with hepatic encephalopathy than the control group. (p<0.05).Of the tested indicators, only albumin was significantly correlated with the clinical grade of hepatic encephalopathy.

The major source of circulating ammonia is the gastrointestinal tract. Due to this reason fasting sample is preferred for the estimation of blood NH₃. Plasma NH₃ concentration in the hepatic portal vein is typically tenfold higher than that in the systemic circulation. It is derived from the action of bacterial proteases, ureases and amines oxidases on the contents of the colon and from the hydrolysis of glutamine in both the small and large intestine. Hence, a protein rich diet cause's marked elevation n blood NH₃ compared to that of the fasting state. The protein in the intestine can be broken down by micro flora into NH₃, indoles, phenols, amine, etc. Also, the urea and creatinine are elevated as a result of reduced glomerular filtration rate (GFR) and decreased tubular function⁸. Retention of these compounds and of metabolic acids is followed by progressive hyperphosphatemia in the patients having uremic encephalopathy.

Also, blood NH₃ was found to be 37.8 ± 4.2 µg/dl in the control group. In hepatic encephalopathy and uremic encephalopathy the level was found to be 142.6 ± 12.5µg/dl and 72.4 ± 11.6 µg/dl respectively. NH₃ showed significant difference between both the hepatic and the uremic encephalopathy patients and control groups. Level of blood NH₃ did not show any correlation with other parameters.

The level of Alkaline Phosphatase did not show any significant changes in either group: Hepatic or Uremic Encephalopathy, as compared to the controls.

Though Serum Creatinine levels showed a significant difference between hepatic encephalopathy and controls, serum Urea levels did not elicit such differences. There was also no significant alteration in Serum calcium between the 2 groups.

In patients with Uremic Encephalopathy, the Chloride and Bicarbonate levels did not show significant alteration when compared to controls.

The levels of serum electrolytes with respect to blood NH₃ has been plotted in Figures 1 and 2.

Fig 1:

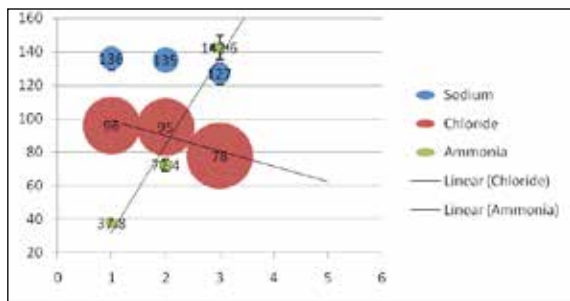


Fig 1 shows that Gradual decrease of serum electrolytes (Sodium, Potassium and Chloride level) in cirrhotic patients favours marked elevation of Plasma NH₃.

Fig. 2:

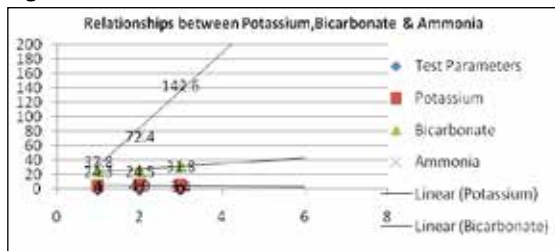


Fig. 2 Represents positive correlations between potassium, bicarbonate and NH₃ level.

Fig. 2 reveals that serum alkaline pH favours pronounced hyperammonia and facilitates more entry of NH₃ into the brain affecting its clearance or utilization process usually taking place in the liver possibly due to dim-

inished activity of various liver mitochondrial enzymes maintaining homeostatic link between Krebs's Urea vs. Krebs's TCA Cycle maintaining both normal Acid-Base balance and urea production simultaneously.

Discussion:

Both hepatic and uremic encephalopathies are severe and common neurological complications in cirrhosis and uremia patients. There are many diagnostic parameters in both forms of encephalopathies; but according to the present study NH₃ along with electrolytes are strongly implicated as a major factor in hepatic encephalopathy only. In the brain, glutamine synthesis is largely confined to astrocytes, and it is now generally believed that excess NH₃ in the brain leads to an excessive accumulation of glutamine in the astrocytic compartment. One postulated mechanism for NH₃ toxicity involves the osmotic effects of glutamine¹. High concentration of glutamine in plasma and the central nervous system are observed in hyperammonemic syndromes, and one study noted that brain glutamine levels are elevated two to four - fold in autopsied brain samples from patients who died with hepatic encephalopathy⁵. Also brain glutamine, or glutamine plus glutamine have been found to be markedly elevated in vivo patients with hyperammonemia⁹.

Blood NH₃ is generated from gastrointestinal update and decomposition of nitrogenous substances in muscle and kidney. Venous NH₃ levels usually depend on factors such as nutritional status, muscle wasting, renal excretion, and the liver urea cycle (which converts NH₃ to urea). Clinical Laboratories usually do not distinguish between ammonium ions (NH₄⁺) and unionized ammonia (NH₃) when determining total venous plasma NH₃ to evaluate the severity of hepatic encephalopathy¹⁰. However, while some NH₃ can cross into the brain as NH₄⁺, unionized NH₃ is uniquely able to freely spread through the blood-brain barrier and cause NH₃ neurotoxicity¹¹.

NH₃ being largely contributed by the intestine is taken almost completely by the liver where it is detoxified to urea. In patients with uremic encephalopathy with normal liver functions, most of the NH₃ is taken by liver. In the kidney small amount of NH₃ is formed by the reaction catalyzed by glutaminase. Still, in the present study, blood NH₃ level is found to be increased significantly in uremic encephalopathy. Generally, all uremic toxins exert their effect through enzyme inhibition, irreversible carbamylation of proteins and derangement in membrane transport¹².

In conclusion, this study supports that serum NH₃ could be useful diagnostic and prognostic tool in cirrhotic patients with hepatic encephalopathy.

Conflict of interest

The authors are declaring no conflict of interest in the present study.

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