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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 encehelopathy, and anions levels, which showed no significant changes in uremic encephalopathy.

KEYWORDS : Hyper Ammonemia, Electrolytes, Encephalopathy

Introduction:

Ammonia (NH_3) has long been known for its poisoning effect on human blood. NH_3 levels fluctuate widely in patients with hepatic encephalopathy, and it is generally accepted that excess NH_3 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 NH_3 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 disease[1]. NH_3 is also implicated in hepatic encephalopathy, where by its toxicity is mediated by glutamine, which is metabolized from NH_3 in the brain[2]. NH_3 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 barrier[3]. Hyper ammonemia may also result in irreversible brain damage[4]. Though NH_3 is toxic, systemic blood NH_3 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 NH_3[5]. In patients with severe liver diseases, impaired renal NH_3 excretion capacity may be a critical factor affecting plasma NH_3. There is a general consensus that gut-derived nitrogenous substances evade hepatic detoxification and affect central nervous system function[6].

NH_3 exists in two forms in blood: ammonia ion (NH_4^+) and unionized ammonia (NH_3). At physiological pH value the pKa for NH_3. Biological membranes are much more permeable to NH_3 than NH_4, so only NH_3 freely passes through the blood-brain barrier. When blood pH increases, the ratio of NH_3/NH_4^+ also increases, and the elevated NH_3 in the brain thus contributes to the cerebral dysfunction associated with hepatic failure[6].

The acquired causes of hyper NH_3 are advanced in liver disease and renal failure. Encephalopathy, is usually taken place both in liver and renal failure, due to hyper NH_3, a highly toxic substances along with glutamate , netrophils 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 NH_3 in the colon leading to hyper NH3. Impaired renal function also causes both uremia and hyperNH_3 having clinical symptoms of nausea, vomiting, malaise, electrolyte imbalance and Acid-Base imbalance.. Hyper NH_3, 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 NH_3 into Glutamate or inhibition of peripheral Glutaminase, actively involved in NH_3 Clearance) in the brain causes Cerebral dysfunction associated with other biochemical abnormalities like Electrolytes and acid-base imbalance. The rate of NH_3 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 benefit[1].

The present study was done to find out the significance of the levels of blood NH_3 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 NH_3, 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 NH_3 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 methodology[7].

Only recently venous samples have been used instead of arterial samples because of case 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 Il,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 continues variables between the groups.

A logic regression analysis was performed to explore the association between blood biochemical variables (NH_3) 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 Glutamyltranferase (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 NH_3. Plasma NH_3 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 NH_3 compared to that of the fasting state. The protein in the intestine can be broken down by micro flora into NH_3, indoles, phenols, amine, etc. Also, the urea and creatinine are elevated as a result of reduced glomerular filtration rate (GFR) and decreased tubular function[8]. Retention of these compounds and of metabolic acids is followed by progressive hyper phosphatemia in the patients having uremic encephalopathy.

Also, blood NH_3 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. NH_3 showed significant difference between both the hepatic and the uremic encephalopathy patients and control groups. Level of blood NH_3 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 Encephelopathy, 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 NH_3 has been plotted in Figures 1 and 2.

Fig 1:

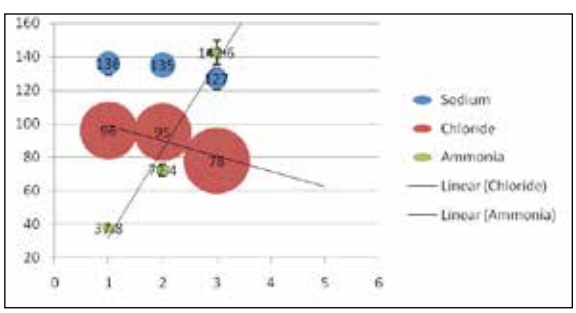

Fig 1 shows that Gradual decrease of serum electrolytes (Sodium, Potassium and Chloride level) in cirrhotic patients favours marked elevation of Plasma NH_3.

Fig. 2:

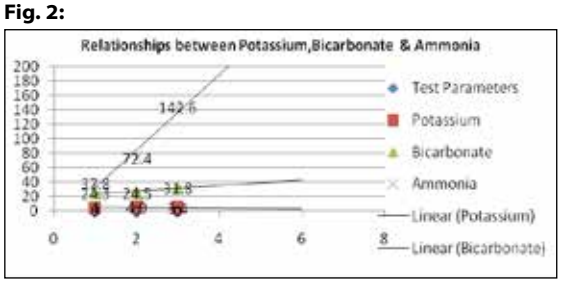

Fig. 2 Represents positive correlationships between potassium, bicarbonate and NH_3 level.

Fig. 2 reveals that serum alkaline pH favours pronounced hyperammonia and facilitates more entry of NH_3 into the brain affecting its clearance or utilization process usually taking place in the liver possibly due to diminished activity of various liver mitochondrial enzymes maintaining homeostatic link between Kreb's Urea vs. Kreb'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 NH_3 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 NH_3 in the brain leads to an excessive accumulation of glutamine in the astrocytic compartment. One postulated mechanism for NH_3 toxicity involves the osmotic effects of glutamine[1]. 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 encephalopathy[5]. Also brain glutamine, or glutamine plus glutamine have been found to be markedly elevated in vivo patients with hyperammonemia[9].

Blood NH_3 is generated from gastrointestinal update and decomposition of nitrogenous substances in muscle and kidney. Venous NH_3 levels usually depend on factors such as nutritional status, muscle wasting, renal excretion, and the liver urea cycle (which converts NH_3 to urea). Clinical Laboratories usually do not distinguish between ammonium ions (NH_4^+) and unionized ammonia (NH_3) when determining total venous plasma NH_3 to evaluate the severity of hepatic encephalopathy[10]. However, while some NH_3 can cross into the brain as NH_4^+, unionized NH_3 is uniquely able to freely spread through the blood -brain barrier and cause NH_3 neurotoxicity[11].

NH_3 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 NH_3 is taken by liver. In the kidney small amount of NH_3 is formed by the reaction catalyzed by glutaminase. Still, in the present study, blood NH_3 level is found to be increased significantly in uremic encephalopathy. Generally, all uremic toxins exert then effect through enzyme inhibition, irreversible carbamoylation of proteins and derangement in membrane transport[12].

In conclusion, this study supports that serum NH_3 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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