

Determination of ammonia concentrations in cirrhosis patients-still confusing after all these years?

Claudia Isabel Blanco Vela,* Francisco Javier Bosques Padilla*

* Servicio de Gastroenterología, Hospital Universitario Dr. José Eleuterio González.
Universidad Autónoma de Nuevo León. Monterrey, Nuevo León, México.

ABSTRACT

By the end of the nineteenth century, ammonia had been identified as the main factor responsible for hepatic encephalopathy syndrome. Ammonia is one of the principal products of hepatic metabolism and high concentrations are toxic to the body. Under physiological conditions, the main way by which the body restricts the blood concentration of ammonia to a nontoxic level is by converting it to urea in the liver via the Krebs cycle. The synthesis of glutamine represents an alternative pathway for ammonia detoxification in cirrhotic patients. Although high concentrations of ammonia have been strongly associated with brain edema, estimates of the strength of the correlation between serum ammonia levels and the severity of hepatic encephalopathy vary. The accuracy of ammonia assays depends on the site of specimen collection, treatment of the specimen and the analytical method used. New methods involving measurement of the partial pressure of ammonia and new noninvasive techniques involving quantification of ammonium in the breath have been described. The purpose of this review is to identify factors that affect serum ammonia levels, from its origin and metabolism to its analysis and interpretation of results in the laboratory. In conclusion, variations in estimates of serum ammonia level and the severity of hepatic encephalopathy arise because of individual differences in ammonia metabolism and differences in the accuracy of analytical methods.

Key words. Hyperammonemia. Hepatic encephalopathy. Hepatic coma. Portosystemic encephalopathy.

INTRODUCTION

The first portacaval anastomosis was performed by Eck in 1877. Sixteen years later, Hahn, *et al.* documented neurological abnormalities in dogs with an Eck fistula that were fed meat. Then, in 1927, Burchi suggested that abnormalities in ammonia metabolism could be the cause of "hepatic coma".¹ Ammonia was subsequently identified as the main factor responsible for hepatic encephalopathy (HE) syndrome. Ammonia is one of the main products of nitrogen metabolism and is converted to urea in the liver. In patients with cirrhosis, the

reduction in hepatocellular function and development of portosystemic shunts contribute to increased serum levels of this neurotoxin. As the gastrointestinal tract is the main site of ammonia production,² reducing the production of ammonia from the intestine was traditionally considered the best therapeutic intervention for treating hyperammonemia. However, studies have shown that other organs are involved in ammonium generation; this has led to the development of new drugs that act systemically and eliminate this compound.³ Although high concentrations of ammonia have been strongly associated with brain edema, estimates of the strength of the correlation between serum ammonia levels obtained in the laboratory and the severity of HE vary. This relationship depends largely on factors associated with the patient such as age, muscle mass, renal function, blood-brain barrier (BBB) permeability and polymorphisms in genes that express enzymes involved in ammonium metabolism, and on variables such as the site and technique used to obtain blood samples, and the analytical quantification method used.

Correspondence and reprint request: Claudia Blanco-Vela, M.D.
Servicio de Gastroenterología.
Hospital Universitario Dr. José Eleuterio González
Francisco I. Madero Pte. s/n y Av. Gonzalitos. Col. Mitras Centro
C.P. 64460, Monterrey, Nuevo León, México.
Tel./Fax: +52 81 8333-3664, +52 81 8348-6068
E-mail: c_i_b_v@hotmail.com

*Manuscript received: May 05, 2010.
Manuscript accepted: May 06, 2010.*

AMMONIA: ORIGIN, METABOLISM AND ELIMINATION

Ammonia is one of the main products of hepatic metabolism and is toxic in high concentrations. Eighty-five percent of the intestinal production of ammonia is generated from deamination of glutamine (Gln) by phosphate-activated glutaminase (PAG) in the small intestine.⁴ PAG catalyzes the hydrolysis of Gln to produce glutamate and ammonia. As this enzyme is present in the liver, kidney, brain and enteric villi, the intestine is not the only site of ammonia production.⁵ Under physiological conditions, the kidney generates ammonia, predominantly via PAG. Thirty percent of the ammonia generated in the kidney by PAG is excreted in the urine, and the remainder enters the systemic circulation through the renal vein.⁶

When the liver is fully functional, the concentration of ammonia is restricted to a nontoxic level by its conversion to urea via the Krebs cycle. Production of ammonia by PAG activity and its removal by urea synthesis take place in the periportal hepatocytes. Ammonia that escapes periportal removal by the urea cycle is removed by perivenous hepatocytes via glutamine synthetase.⁷ Gln synthesis represents an alternative means of ammonia detoxification in cirrhotic patients.⁸ Gln acts as a nontoxic ammonium transporter in the circulation.

In patients with cirrhosis, the reduction in hepatocellular function and the generation of portosystemic shunts contribute to increased serum levels of this neurotoxin.⁹ In this context, high blood ammonia levels may be an indirect measure of the presence of collateral blood vessels and esophageal varices, and could help to identify cirrhotic patients who require closer endoscopic follow-up.¹⁰ When hepatic activity is insufficient to maintain ammonia homeostasis, other organs are adapted to respond to this situation. In patients with cirrhosis, the ammonia that cannot be converted to a nontoxic compound in the liver is released into the systemic circulation and is taken up by muscle.¹¹ Muscle constitutes more than one-third of body weight and therefore is one of the main sources of Gln.¹² Because of the activity of glutamine synthetase and protein catabolism. As the Gln produced by muscle may be released and subsequently used as a substrate for the generation of ammonia in the intestine and kidney, its net effect on systemic ammonium mobilization is limited.¹³

The kidneys make the main contribution to balancing serum ammonia levels. In hyperammonemia,

the relationship between generation and renal clearance of ammonia is altered such that the release of ammonia into the circulation by the renal vein is decreased and its urinary excretion is increased,¹⁴ as occurs in response to metabolic acidosis, hypokalemia and extracellular volume expansion.¹⁵ The increase in the renal elimination of ammonia in the urine stimulates the generation of bicarbonate, which in turn can be used in the hepatic synthesis of urea.¹⁶ Jalan, *et al.* showed that saline infusion decreases plasma ammonia level from 93 $\mu\text{mol/L}$ to 56 $\mu\text{mol/L}$ ($p < 0.05$) and increases urinary excretion of ammonia from 0.9 mmol/h to 1.4 mmol/h ($p < 0.05$).¹⁵ It follows that, in patients with acute HE, a favorable clinical outcome will be obtained in response to hydration and that the kidney should be the target organ for new therapeutic modalities.

Ammonia has been strongly related to brain edema secondary to liver failure.¹⁷ A histological study of patients who died of HE revealed evidence of edema of astrocytes, neural cells considered most sensitive to hepatic failure.¹⁸ Glutamate is the major excitatory neurotransmitter in the central nervous system. Reuptake of glutamate by the astrocyte prevents its accumulation in the synaptic space and protects neurons from excessive activation. Within the astrocyte, glutamate is a precursor of Gln and glutathione. Gln synthesis is the main mechanism responsible for ammonia detoxification in the central nervous system.¹⁹ Glutamine synthetase activity in the brain is confined to astrocytes. Gln is released into the extracellular space and is taken up by neurons to produce glutamate and ammonium, a reaction mediated by PAG. Both HE and the hyperammonemic states are associated with elevated levels of Gln in the brain and spinal fluid.

As Gln has an osmotic effect, high levels could cause cellular edema.^{20,21} In response to accumulation of Gln and the resultant hypotonicity, glial cells release osmolytes such as myoinositol and taurine into the extracellular space. This partially restores intracellular osmolarity but does not prevent subsequent cell swelling.

In cirrhosis patients, dilutional hyponatremia is a typical response to inappropriate antidiuretic hormone secretion. Hyponatremia has been identified as an independent risk factor for HE.²² During hyponatremia and edema, cerebral osmolytes are released into the extracellular space. A decrease in the intracellular level of osmolytes during hyponatremia may limit the ability of astrocytes to adapt to the increase in intracellular osmolality caused by Gln in hyperammonemic states.²³

As small changes in the extracellular environment of the brain can affect neurotransmission, the BBB, which is composed of endothelial cells connected by tight junctions, limits the entrance of substances that are potentially harmful for the central nervous system. In the past, it was believed that ammonia was unable to pass through the BBB because it is impermeable to ions, and ammonia is mainly present in the ionized form, NH_4^+ , in the systemic circulation. However, Lockwood, *et al.* showed that ^{13}N -labeled ammonia was able to cross the BBB. Ammonia that passes through the BBB is used by the brain and its utilization rate increases in parallel with arterial ammonia concentration. Cerebral metabolic rate is elevated in patients with portosystemic encephalopathy and is accompanied by an increase in permeability per unit surface area, a measure of BBB permeability.¹¹

Astrocytes are a component of the BBB and regulate the brain's blood flow via an arachidonic acid-dependent pathway.²⁴ As presence of markers of the systemic inflammatory response is a predictor of deterioration in the degree of HE, it is assumed that inflammation exacerbates the neurological changes induced by hyperammonemia.²⁵ This may be the result of disruption of the BBB during acute infectious episodes.²⁶ Although treatment with nonsteroidal anti-inflammatory drugs has shown improvement in cognitive function in animal models of portosystemic encephalopathy,²⁷ their use in humans has been limited because of concerns about their effect on kidney function.

The magnitude of the clinical manifestations of a sudden increase in serum ammonia level differs from one patient to another. These differences may result from genetic alterations in the key enzymes of Gln metabolism. Using experimental animal models, several polymorphisms in the promoter of the PAG gene have been identified which may explain variation in the activity of this enzyme in humans.²⁸ The intestinal activity of PAG is elevated in patients with cirrhosis and is indicative of minimal HE.²⁹ Studies on polymorphisms in the PAG gene have revealed that the TACC haplotype is associated with improved liver function, reduced risk of HE and low intestinal ammonia production.

The oral Gln challenge is a method that increases blood ammonia concentration and is an indirect measure of intestinal PAG activity.³⁰ In patients with minimal HE, a positive oral Gln challenge result is associated with a 60% risk of an episode of acute HE within one year.³¹ The oral Gln load affects the results of neuropsychological and neuro-

physiological tests, especially in patients with EEG abnormalities or minimal HE, indicating that cirrhotic patients become sensitized to ammonia.³²

Hypersensitivity to this neurotoxin could be mediated by cell membrane water channels called aquaporins. These channels regulate the flow of water to and from the brain and are intimately involved with the development of cerebral edema in patients with cirrhosis. Hyperammonemia upregulates aquaporin 4 expression. Increased aquaporin expression results in osmotic gradients across cell membranes. The precise regulatory mechanisms of these proteins have not been clarified, but they are probably responsible for astrocyte edema and increased BBB permeability in liver failure.³³

DETERMINATION OF AMMONIA

The ammonia concentrations of cirrhosis patients have been measured since the beginning of last century, and it has been observed that ammonia concentrations are affected by factors other than the analytical method used. Ammonia levels are four to eight times higher in neonates than in adults, two to three times higher in children under the age of three years than in adults, and are commensurate with those of adults during adolescence.³⁴ Exercise increases the level of ammonia by up to three times, and the increase is highest among men.³⁵ Ammonia level increases by $10 \mu\text{mol/L}$ after smoking a cigarette.³⁶ The absorption of glycine during transurethral prostatectomy can cause a transient elevation in ammonia level and induce metabolic encephalopathy.³⁵ The administration of valproic acid is associated with hyperammonemia, especially in patients with carnitine deficiency.³⁷ Idiopathic hyperammonemia syndrome occurs when abnormalities in liver function are absent, and has been associated with the use of chemotherapy after bone marrow transplantation.³⁸

Ammonia concentration is affected by the site from which the specimen is collected, the way in which the specimen is processed and the analytical method used. If a blood sample is centrifuged immediately and the plasma is refrigerated at 4°C , ammonia concentration will remain relatively stable for up to 60 min, and the increase in ammonia concentration will be limited to $2 \mu\text{mol/L}$ (5%).³⁹ In patients with liver disease, centrifugation of the sample within 15 min of collection is ideal.⁴⁰ The sample should be placed on ice immediately after collection because erythrocyte and platelet metabolism persist *in vitro* and may increase ammonia

concentration by 20% within 1 h and by up to 100% within 2 h if the sample is held at room temperature. There is a positive relationship between the rate of ammonia formation *in vitro* and the subject's level of alanine aminotransferase (ALT).⁴¹

In 1963, Stahl reported that arterial blood was most representative of whole body ammonia levels because the concentration of ammonia in venous blood may be affected by peripheral metabolism and the uptake of ammonia by muscle and brain tissue.⁹ In biological fluids, ammonia exists in two forms: as ionized ammonium, NH_4^+ , and in its gaseous unionized form, NH_3 . At a physiological pH of 7.4, 98% of plasma ammonia is in the ionized form.⁴² The level of NH_3 , which freely crosses the BBB, is a function of the partial pressure of ammonia in the blood. Kramer, *et al.* measured the arterial partial pressures of NH_3 gas and total arterial ammonia concentrations in patients with acute HE (grades I–IV).⁴³ They found that the clinical grade of encephalopathy was better correlated with the partial pressure of NH_3 than with total arterial ammonia concentrations ($r = 0.79$ vs. $r = 0.69$, respectively; $p = 0.01$). Subsequently, Ong, *et al.* compared arterial and venous ammonia concentrations with the partial pressure of NH_3 in arterial and venous blood.⁴⁴ Results obtained using these four approaches were all significantly correlated with the West Haven scale ($p \leq 0.001$). The correlation coefficients were arterial total blood ammonia, $r = 0.61$; venous blood ammonia concentration, $r = 0.56$; arterial partial pressure of NH_3 , $r = 0.55$; and venous partial pressure of NH_3 , $r = 0.52$. It is important to note that the population analyzed by Kramer was composed entirely of patients with acute HE, and that analyzed by Ong, *et al.* was composed of cirrhotic patients with or without clinically manifest HE. This difference could have caused the difference in their estimates of the correlation coefficient between grade of encephalopathy and arterial partial pressure of NH_3 ($r = 0.79$ vs. $r = 0.55$, respectively).

Several efforts have been made to develop more sensitive and less invasive specimen collection techniques. Dubois, *et al.* tested the efficacy of measuring ammonia concentrations in expired breath using an optic fiber sensor on 17 cirrhosis patients, and reported a negative correlation between breath test results and the time required to complete the number connection test ($r = -0.55$, $p = 0.03$).⁴⁵

An attractive alternative method for measuring ammonia concentration involves a pocket device that enables bedside specimen processing. The pocket analyzer is a portable device for determining

ammonia concentration in whole blood. It requires a small blood sample (20 mL) and produces results within 3 min. The immediate analysis of the sample decreases error arising from spontaneous generation of ammonia by *in vitro* metabolism of erythrocytes. This method is based on microdiffusion and colorimetry. The lower and upper detection limits of the unit are 7 $\mu\text{mol/L}$ and 286 $\mu\text{mol/L}$, respectively. Goggs, *et al.* compared the accuracy of the pocket analyzer with that of an enzymatic analytical method,⁴⁶ and found that results obtained using the pocket device were positively correlated with the reference method (intraclass correlation coefficient, 0.800; 95% confidence interval, 0.655–0.888). The pocket analyzer has a proportional negative bias, i.e., it underestimates ammonia level to a greater extent at high concentrations than at low concentrations.

The most widely used analytical method for detecting ammonium is an enzymatic method. This analysis is based on the reaction of glutamate dehydrogenase, which catalyzes the condensation of NH_4 and 2-oxoglutarate to form glutamate in the presence of NADH or NADPH. The concentration of ammonia is proportional to the oxidation of NADPH to NADP, the concentration of which is measured as the change in absorbance at 340 nm using a spectrophotometer. High levels of ALT in serum may interfere with this measurement. Pyruvate is a by-product of ALT activity and is reduced by NADH to lactate in the presence of lactate dehydrogenase (present in the ammonia reagent), which increases the amount of NAD in the solution, resulting in an overestimation of ammonia concentration.⁴⁷

The dry chemistry method uses a reactive multi-layer incorporated into a polyester base. Ammonia reacts with a bromophenol blue indicator to produce a colored compound, which is measured by reflectance spectrophotometry at 600 nm. This method is safer, simpler and less expensive than the enzymatic method and produces results within 5 min.⁴⁸

Herrera, *et al.* compared the enzymatic and dry chemistry methods in patients with clinical conditions such as acute liver failure or multiorgan failure, which are associated with high or moderate elevation of ALT level.⁴⁷ They added solutions with progressively elevated concentrations of ALT to plasma samples to assess the effect of ALT on ammonia concentration. With the enzymatic method, the addition of ALT elevated ammonium concentration by 96 $\mu\text{mol/L}$ to 391 $\mu\text{mol/L}$. The activities of other dehydrogenases present in the sample could be responsible for these differences. Two-step enzymatic methods are available that enable corrections to be

made to allow for nonspecific oxidation of NADPH to NAD, which generates results similar to those obtained using the dry chemistry method.⁴⁹

CONCLUSIONS

The availability of more sensitive and specific analytical methods has increased the diagnostic accuracy of blood ammonia concentrations. We suggest the use of the dry chemistry or the two-step enzymatic method to measure ammonia. Although improvements in analytical methods have resulted in stronger correlations between hyperammonemia and the severity of HE, there is still considerable variation between these estimates, possibly because of interobserver variability in assessing the severity of HE. Neuropsychological tests are influenced by the patient's age and education level. The main difficulty is that there is no standard definition of HE in neuropsychological terms. Therefore, it must be recognized that the sensitivities of methods for clinical evaluation of HE are such that concordant results from more than one method are required before a diagnosis of HE can be made with confidence.

We recommend using blood ammonia concentrations:

- In conjunction with the results of clinical, neuropsychological and neurophysiological tests.
- To assess the effectiveness of drugs for research purposes.
- To monitor fluctuations that may occur in an individual patient, as there is no threshold of ammonia level for cognitive impairment.

Changes in a patient's ammonia concentration may be a predictor of the onset of clinical manifestations. The disparity between serum ammonia levels and clinical severity probably resides in failure to take into account the influence of systemic ammonia metabolism, individual genetic variations in the expression of enzymes involved in Gln metabolism and cell membrane proteins associated with regulation of osmotic gradients in the brain, and the role of other unidentified neurotoxic substances.

ABBREVIATIONS

- **EH:** Hepatic encephalopathy.
- **BBB:** Blood-brain barrier.
- **PAG:** Phosphate-activated glutaminase.
- **Gln:** Glutamine.
- **ALT:** Alanine aminotransferase.

REFERENCES

1. McDermott W. The role of ammonia intoxication in hepatic coma. *Bull N Y Acad Med* 1958; 34(6): 357-65.
2. McDermott WV Jr. Metabolism and toxicity of ammonia. *N Engl J Med* 1957; 257: 1076-81.
3. Olde DSW, Jalan R, Dejong CH. Interorgan ammonia trafficking in liver disease. *Metab Brain Dis* 2009; 24(1): 169-81.
4. Huizenga JR, Gips CH, Tangerman A. The contribution of various organs to ammonia formation: a review of factors determining the arterial ammonia concentration. *Ann Clin Biochem* 1996; 33: 23-30.
5. James LA, Lunn PG, Middleton S, Elia M. Distribution of glutaminase and glutamine synthetase activities in the human gastrointestinal tract. *Clin Sci* 1998; 94(3): 313-9.
6. Halperin ML, Kamel KS, Ethier JH, et al. Biochemistry and physiology of ammonia excretion. In: Seldin DW, Giebisch G (eds.). *The Kidney: Physiology and Pathophysiology*. New York: Raven Press Ltd.; 1992, p. 2645-79.
7. Häussinger D. Nitrogen metabolism in liver: structural and functional organization and physiological relevance. *Biochem J* 1990; 267(2): 281-90.
8. Romero-Gómez M. Role of phosphate-activated glutaminase in the pathogenesis of hepatic encephalopathy. *Metab Brain Dis* 2005; 20(4): 319-25.
9. Stahl J. Studies of the blood ammonia in liver disease. Its diagnostic, prognostic, and therapeutic significance. *Ann Intern Med* 1963; 58: 1-24.
10. Tarantino G, Citro V, Esposito P, Giaquinto S, De Leone A, Milan G, et al. Blood ammonia levels in liver cirrhosis: a clue for the presence of portosystemic collateral veins. *BMC Gastroenterol* 2009; 9: 21.
11. Lockwood AH, McDonald JM, Reiman RE, Gelbard AS, Laughlin JS, Duffy TE, Plum F. The dynamics of ammonia metabolism in man. Effects of liver disease and hyperammonemia. *J Clin Invest* 1979; 63(3): 449-60.
12. Morgan KZ, Binks W, Brues AM, Cipriani AJ, Langham WH, Marinelli LD, et al. Report of International Subcommittee II on permissible dose for internal radiation. *Br J Radiol Suppl* 1955; 6: 25.
13. Olde DSW, Jalan R, Redhead DN, Hayes PC, Deutz NE, Soeters PB. Interorgan ammonia and amino acid metabolism in metabolically stable patients with cirrhosis and a TIPSS. *Hepatology* 2002; 36(5): 1163-71.
14. Dejong CH, Deutz NE, Soeters PB. Renal ammonia and glutamine metabolism during liver insufficiency-induced hyperammonemia in the rat. *J Clin Invest* 1993; 92(6): 2834-40.
15. Jalan R, Kapoor D. Enhanced renal ammonia excretion following volume expansion in patients with well compensated cirrhosis of the liver. *Gut* 2003; 52(7): 1041-5.
16. Meijer AJ, Lamers WH, Chamuleau RAFM. Nitrogen metabolism and ornithine cycle function. *Physiol Rev* 70: 701-48.
17. Ranjan P, Mishra A, Kale R, Saraswat V, Gupta R. Cytotoxic edema is responsible for raised intracranial pressure in fulminant hepatic failure: in vivo demonstration using diffusion-weighted MRI in human subjects. *Metab Brain Dis* 2005; 20(3): 181-92.
18. Gibson JB. Encephalopathy after portacaval shunt. *Br Med J* 1963; 1: 1652-55.
19. Hazell A, Butterworth R. Hepatic encephalopathy: an update of pathophysiological mechanisms. *Exp Biol Med* 1999; 222: 99-112.
20. Benarroch E. Neuron-astrocyte interactions: partnership for normal function and disease in the central nervous system. *Mayo Clin Proc* 2005; 80(10): 1326-38.

21. Norenberg MD, Rao KV, Jayakumar AR. Mechanisms of ammonia-induced astrocyte swelling. *Metab Brain Dis* 2005; 20(4): 303-18.
22. Guevara M, Baccaro ME, Torre A, Gómez-Ansón B, Ríos J, Torres F, et al. Hyponatremia is a risk factor of hepatic encephalopathy in patients with cirrhosis: a prospective study with time-dependent analysis. *Am J Gastroenterol* 2009; 104(6): 1382-9.
23. Heins J, Zwingmann C. Organic osmolytes in hyponatremia and ammonia toxicity. *Metab Brain Dis* 2010; 25(1): 81-9.
24. Takano T, Tian GF, Peng W, Lou N, Libionka W, Han X, et al. Astrocyte-mediated control of cerebral blood flow. *Nat Neurosci* 2006; 9: 260-7.
25. Shawcross DL, Davies NA, Williams R, Jalan R. Systemic inflammatory response exacerbates the neuropsychological effects of induced hyperammonemia in cirrhosis. *J Hepatol* 2004; 40(2): 247-54.
26. Vaquero J, Polson J, Chung C, Helenowski I, Schiodt FV, Reisch J, et al. Infection and the progression of hepatic encephalopathy in acute liver failure. *Gastroenterology* 2003; 125(3): 755-64.
27. Cauli O, Rodrigo R, Piedrafita B, Boix J, Felipe V. Inflammation and hepatic encephalopathy: ibuprofen restores learning ability in rats with portacaval shunts. *Hepatology* 2007; 46(2): 514-9.
28. Taylor L, Liu X, Newsome W, Shapiro RA, Srinivasan M, Curthoys NP. Isolation and characterization of the promoter region of the rat kidney-type glutaminase gene. *Biochim Biophys Acta* 2001; 1518: 132-6.
29. Romero-Gómez M, Ramos-Guerrero R, Grande L, de Teran LC, Corpas R, Camacho I, Bautista JD. Intestinal glutaminase activity is increased in liver cirrhosis and correlates with minimal hepatic encephalopathy. *J Hepatol* 2004; 41: 49-54.
30. Romero-Gómez M, Jover M, Galán JJ, Ruiz A. Gut ammonia production and its modulation. *Metab Brain Dis* 2009; 24(1): 147-57.
31. Romero-Gómez M, Grande L, Camacho I, Benitez S, Irlés JA, Castro M. Altered response to oral glutamine challenge as prognostic factor for overt episodes in patients with minimal hepatic encephalopathy. *J Hepatol* 2002; 37: 781-7.
32. Masini A, Efrati C, Merli M, Nicolao F, Amodio P, Del Piccolo F, Riggio O. Effect of blood ammonia elevation following oral glutamine load on the psychometric performance of cirrhotic patients. *Metab Brain Dis* 2003; 18(1): 27-35.
33. Rama RKV, Chen M, Simard JM, Norenberg MD. Increased aquaporin-4 expression in ammonia-treated cultured astrocytes. *NeuroReport* 2003; 14: 2379-82.
34. Diaz J, Tornel PL, Martinez P. Reference intervals for blood ammonia in healthy subjects, determined by micro-diffusion [Letter]. *Clin Chem* 1995; 41: 1048.
35. Derave W, Bouckaert J, Pannier JL. Gender differences in blood ammonia response during exercise. *Arch Physiol Biochem* 1997; 105: 203-9.
36. Huizenga JR, Tangerman A, Gips CH. Determination of blood ammonia in biological fluids. *Ann Clin Biochem* 1994; 31: 529-43.
37. Shepard RL, Kraus SE, Babayan RK, Siroky MB. The role of ammonia toxicity in the post transurethral prostatectomy syndrome. *Br J Urol* 1987; 60(4): 349-51.
38. Davies SM, Szabo E, Wagner JE, Ramsay NK, Weisdorf DJ. Idiopathic hyperammonemia: a frequently lethal complication of bone marrow transplantation. *Bone Marrow Transplant* 1996; 17(6): 1119-25.
39. Howanitz JH, Howanitz PJ, Skrodzki CA, Iwanski JA. Influences of specimen processing and storage conditions on results for plasma ammonia. *Clin Chem* 1984; 30: 906-8.
40. Dufour DR, Lott JA, Nolte FS, Gretch DR, Koff RS, Seeff LB. Diagnosis and monitoring of hepatic injury. I. Performance characteristics of laboratory tests. *Clin Chem* 2000; 46(12): 2027-49.
41. Da Fonseca-Wollheim F. Preanalytical increase of ammonia in blood specimens from healthy subjects. *Clin Chem* 1990; 36(8, Pt. 1): 1483-7.
42. Warren KS. Ammonia toxicity and pH. *Nature* 1962; 195: 47-9.
43. Kramer L, Tribl B, Gendo A, Zauner C, Schneider B, Ferenci P, Madl C. Partial pressure of ammonia versus ammonia in hepatic encephalopathy. *Hepatology* 2000; 31(1): 30-4.
44. Ong JP, Aggarwal A, Krieger D, Easley KA, Karafa MT, Van Lente F, et al. Correlation between ammonia levels and the severity of hepatic encephalopathy. *Am J Med* 2003; 114(3): 188-93.
45. DuBois S, Eng S, Bhattacharya R, Rulyak S, Hubbard T, Putnam D, Kearney DJ. Breath ammonia testing for diagnosis of hepatic encephalopathy. *Dig Dis Sci* 2005; 50(10): 1780-4.
46. Goggs R, Serrano S, Szladovits B, Keir I, Ong R, Hughes D. Clinical investigation of a point-of-care blood ammonia analyzer. *Vet Clin Pathol* 2008; 37(2): 198-206.
47. Herrera DJ, Moore S, Heap S, Preece MA, Griffiths P. Can plasma ammonia be measured in patients with acute liver disease? *Ann Clin Biochem* 2008; 45(4): 426-8.
48. Iosefsohn M, Hicks JM. Ektachem multilayer dry-film assay for ammonia evaluated. *Clin Chem* 1985; 31(12): 2012-4.
49. Herrera DJ, Hutchin T, Fullerton D, Gray G. Non-specific interference in the measurement of plasma ammonia: importance of using a sample bank. *Ann Clin Biochem* 2010; 47(1): 81-3.