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A simple and rapid method for determining blood ammonia levels.

Tetsuya Shiota[*] Akiharu Watanabe[†] Nobuyuki Takei[‡]

Shosaku Hayashi[**] Takahiro Obata[††]

Tatsuro Sakata[‡‡] Hideo Nagashima[§]

[*]Okayama University,
[†]Okayama University,
[‡]Okayama University,
[**]Okayama University,
[††]Okayama University,
[‡‡]Okayama University,
[§]Okayama University,

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Tetsuya Shiota, Akiharu Watanabe, Nobuyuki Takei, Shosaku Hayashi, Takahiro Obata, Tatsuro Sakata, and Hideo Nagashima

Abstract

Blood ammonia levels in patients with various liver diseases were determined quantitatively by a simple and rapid method using the Amitest Meter System. The results were compared to those obtained by an enzymatic method and were well correlated. This simple Amitest is also useful in animal experiments, particularly when there is a need to determine blood ammonia levels serially. This paper test was evaluated as being accurate and reliable for clinical and experimental use.

KEYWORDS: simple method, blood ammonia, hyperammonemia, liver disease

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— BRIEF NOTE —

A SIMPLE AND RAPID METHOD FOR DETERMINING BLOOD AMMONIA LEVELS

Tetsuya SHIOTA, Akiharu WATANABE, Nobuyuki TAKEI, Shosaku HAYASHI, Takahiro OBATA, Tatsuro SAKATA and Hideo NAGASHIMA

First Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan

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Abstract. Blood ammonia levels in patients with various liver diseases were determined quantitatively by a simple and rapid method using the Amitest Meter System. The results were compared to those obtained by an enzymatic method and were well correlated. This simple Amitest is also useful in animal experiments, particularly when there is a need to determine blood ammonia levels serially. This paper test was evaluated as being accurate and reliable for clinical and experimental use.

Key word: simple method, blood ammonia, hyperammonemia, liver disease.

Determination of blood ammonia levels is clinically important when predicting the development of hepatic encephalopathy in severe liver disease and when judging the efficacy of treatment during encephalopathy. Detection of hyperammonemia is also indispensable in the field of pediatrics for diagnosing congenital enzyme defects in the urea cycle. Many procedures for determining blood ammonia levels have already been reported, for example, microdiffusion analysis (1, 2), a direct colorimetric procedure (3), an ion exchange technique (4) and an enzymatic method (5). Some of these are time-consuming techniques using special apparatus.

Tada *et al.* (6, 7) recently reported a screening paper test based on the principle of microdiffusion of ammonia. We have already investigated this semi-quantitative method and found that the simple and rapid test was reliable and valuable for blood ammonia concentrations at the bedside. The method, however, only shows blood ammonia levels every fifty nitrogen (N)-μg/dl and is not enough for following accurately variations of blood ammonia levels during the clinical course. These results led us to quantitate the levels more accurate not only for clinical but also for experimental materials using the Amitest Meter System.

Materials and methods. Determination of blood ammonia levels was performed in 18 patients with various liver diseases, such as liver cirrhosis, hepatocellular carcinoma and idiopathic portal hypertension. Venous blood was collected in the

early morning without using anticoagulant, and ammonia levels were measured immediately using the Ammonia Reagent Plate and the Amitest Meter. This blood ammonia analyzer is manufactured and sold by Kyoto Daiichi Kagaku Co., Ltd. (57-Nishiakeda-cho, Higashikujo, Minami-ku, Kyoto, 601, Japan). An enzymatic kit method was performed using Determiner-NH_3 (Kyowa Hakko Co., Ltd., Tokyo, 100, Japan) (5). Ammonium sulfate was added to blood specimens with known values of ammonia to investigate the recovery of ammonia from a sample. Blood ammonia levels of two patients with liver cirrhosis were measured ten times, respectively, to investigate coefficients of variation. After blood specimen was diluted by physiological saline, blood ammonia levels were measured to investigate the effect of hemoglobin concentrations. Interference with blood ammonia levels by amino acids, urea, uric acid and bilirubin was investigated by adding these substances to blood specimens. Amino acid mixture was obtained by drying commercially available 12 % amino acid solution.

Ammonium acetate was administered at a dose of 2.7 mmoles per kg body weight into the large intestine after laparotomy in male Sprague-Dawley rats weighing 220 and 350 g. Blood was drawn from a cervical vein using a small syringe before and 10, 30, 60 and 120 min after ammonium acetate administration. Ammonia determinations were carried out after obtaining the blood samples without using anticoagulant, as described above.

Results and discussion. Blood ammonia levels of various liver diseases measured by the Amitest Meter System were compared with those by an enzymatic method. The levels determined by these two procedures correlated well ($r = 0.979$, $p < 0.001$, $y = 1.03x + 0.017$). Ammonia levels were slightly underestimated and overestimated when measured before and after 15 min of the reaction time, respectively (for example, 150 N-μg/dl in 15 min, 135 in 13 min and 158 in 18 min). The reaction was considered to be adequate (Table 1). Coefficients of variation of blood ammonia levels of two patients with liver cirrhosis were 4.31 and 2.81 %, and mean and standard deviations, 52.0 ± 2.2 and 175.2 ± 4.9 N-μg/dl, respectively. The difference in ammonia levels is very little within the physiological range of hemoglobin concentrations from 5.5 to 15.0 g/dl. Interference with blood

TABLE 1. RECOVERY EXPERIMENT OF BLOOD AMMONIA LEVELS USING THE AMITEST METER SYSTEM

Ammonium sulfate added (N-μg/dl)	Ammonia value (N-μg/dl)		Recovery (%)
	Expected	Measured	
0		21	
50	71	73	102.8
100	121	131	108.3
200	221	232	105.0
300	321	309	96.2

ammonia levels by amino acid mixture, urea, uric acid and bilirubin in the blood was negligible within clinically expected alterations up to 120 mg/dl of amino acid mixture, 100 mg/dl of urea, 20 mg/dl of uric acid and 50 mg/dl of bilirubin.

Using this simple and rapid method at the bedside, good relation between clinical symptoms and blood ammonia levels was observed in a patient with liver cirrhosis (58-year-old man) by measuring the levels for three times (Fig. 1). Flapping tremor, ascites and jaundice were seen with hyperammonemia on admission. Treatment for hyperammonemia such as lactulose enema and oral administration

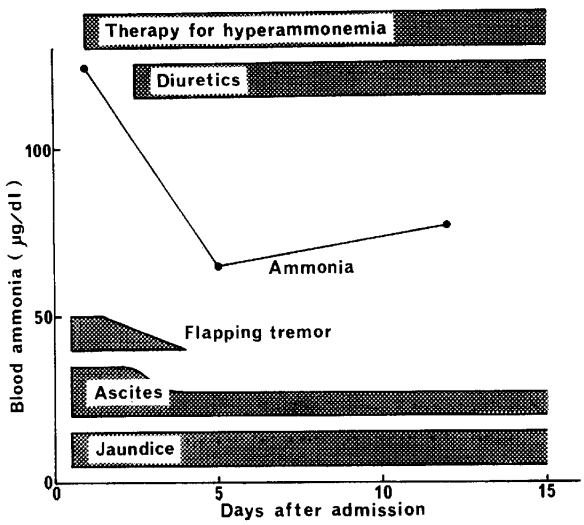

Fig. 1. Clinical course of a case with liver cirrhosis. Blood ammonia levels in 58-year old man were followed up by the Amitest Meter System.

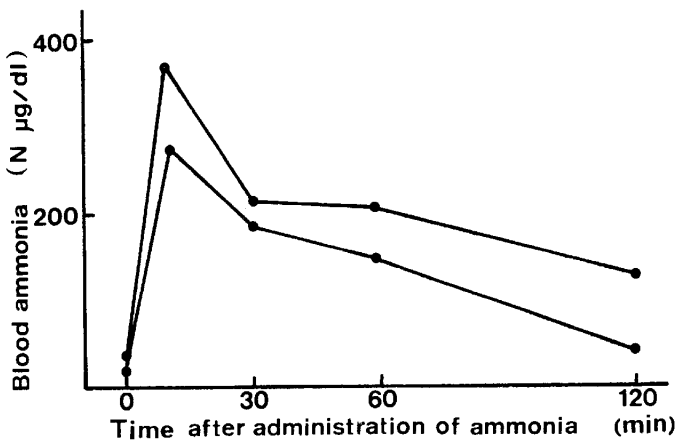

Fig. 2. Changes in blood ammonia levels following ammonium acetate loading to rats. Other experimental details are described under Materials and methods.

of nonabsorbable antibiotics lowered ammonia levels in proportion to improvement in the neurological symptoms.

Ammonia was loaded into the colon of rats fasted overnight and blood ammonia levels were determined frequently thereafter (Fig. 2). The maximam levels of blood ammonia was observed 15 min following intestinal administration of ammonia. The levels gradually declined thereafter and tended to return the basal levels.

Determination of blood ammonia levels is clinically important to detect hyperammonemia in various diseases. Blood ammonia levels can be measured very easily by this system without any special technique, and it takes about only 20 min from sampling the blood specimen to displaying the level. Serial and frequent determinations, which were not easy in the past, are also possible. Although this simple method is essentially the same as the screening paper test (6, 7), because of the digital display from 1 to 400 N-μg/dl, quantitative changes in the levels of blood ammonia can be followed accurately and easily during the clinical course. Also in animal experiments, this simple method can be applied to investigate variations in the levels frequently. From these aspects, no other method of blood ammonia determination would be superior to the Amitest Meter System.

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