ACCURACY OF A POINT-OF-CARE AMMONIA ANALYZER FOR SCREENING OF BLOOD AMMONIA IN PEDIATRIC PATIENTS WITH INBORN ERROR OF METABOLISM

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Abstract. Hyperammonemia is an emergency condition that can be life-threatening in pediatric patients. However, the laboratories in most general hospitals in Thailand do not have the testing equipment needed to measure blood ammonia concentration. A point-of-care (POC) ammonia analyzer has recently been introduced that facilitates fast and easy measurement of blood ammonia concentration. To validate the accuracy of this device, we conducted a cross sectional study to compare blood ammonia concentrations measured by the new POC device with concentrations measured by standard enzymatic method in patients with known or suspected inborn errors of metabolism. A total of 20 blood samples were collected from 14 pediatric patients. Scatter plot of blood ammonia concentrations from the POC device and the enzymatic method demonstrated a strong positive correlation, with a Pearson's correlation coefficient of 0.95 ($p<0.0001$) and an intraclass correlation coefficient of 0.93 (95% CI: 0.811-0.974). Bland-Altman plot revealed a mean difference of -7.3 µmol/l between ammonia concentration measurements from the POC device and the enzymatic method. Using a standard cutoff value, ammonia concentration measurements were in satisfactory agreement in 18 of 20 (90%) samples. Transportation of samples within 30 minutes and EDTA-containing samples did not significantly affect blood ammonia concentrations. In conclusion, POC ammonia testing may be a suitable method for hyperammonemia screening in general hospitals where blood ammonia concentration measurement by standard enzymatic method is not available.

Keywords: point-of-care testing, POC, ammonia, hyperammonemia, inborn errors of metabolism

INTRODUCTION

Hyperammonemia is an emergency condition in medicine. Ammonia is a potent toxin that most notably adversely affects central nervous system (CNS) functions. Hyperammonemia can affect patients at any age, and can be classified as either primary or secondary hyperammonemia (Haberle, 2011). Primary hyperammonemia is diagnosed in patients with inherited defects of urea cycle enzymes or transporters; whereas, secondary hyperammonemia is diagnosed in patients with inhibition of urea cycle by other inborn errors of metabolism, such as organic acidemias, drugs (*eg*, valproate), and inherited or acquired liver dysfunction. Although hyper-ammonemia can be life-threatening, its signs and symptoms are not specific – especially in neonates and young children (Summar, 2001). Clinical presentations of this condition include

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poor feeding, lethargy, vomiting, headache, behavioral change, seizures leading to coma, and death (Burton, 1998). As such, the presence of hyperammonemia requires prompt clinical recognition, confirmation by measurement of blood ammonia concentration, and treatment in order to prevent disability and mortality. However, the majority of laboratories in general hospitals in Thailand are not able to measure blood ammonia concentration (Vatanavichan, personal communication). This diagnostic limitation leads to delays in diagnosis of hyperammonemia in Thai children with inborn errors of metabolism (Vatanavicharn *et al*, 2012).

Most of the biochemical laboratories in the world currently use enzymatic assay to measure blood ammonia concentration. This method is based on reductive amination of 2-oxoglutarate with glutamate dehydrogenase (GLDH) and reduced nicotinamide adenine dinucleotide phosphate (NADPH): 2-oxoglutarate + NH_3 + NADPH $\xrightarrow{\text{GLDH}}$ glutamate + NADP. Decrease in absorbance at 340 nm caused by the oxidation of NADPH is proportional to blood ammonia concentration (van Anken and Schiphorst, 1974; Barsotti, 2001). This method can be used to measure a broad range of ammonia concentrations, but the disadvantages of this assay are its turnaround time (TAT) and the complexity of the procedure. A new measurement technique was then developed that uses microdiffusion of released free ammonia by alkalization with a colorimetric assay (Huizenga *et al*, 1992). This method is simpler and has a faster TAT (<4 minutes), which makes it suitable as a bedside procedure. A point-of-care (POC) device that is based on this methodology was then developed and tested in both animal and human studies. Several studies tested this device and reported reliable results, and improved diagnosis and monitoring of hyperammonemia (de Keijzer *et al*, 1997; Goggs *et al*, 2008; Ditisheim *et al*, 2011). Although this POC device was adopted as an acceptable substitute method for detection of hyperammonemia in Thailand, the accuracy of this POC device and the factors that could influence its reliability in a Thai healthcare setting have not been established. Moreover, the average temperature in Thailand ranges from 30-36°C, and this factor could affect POC ammonia concentration measurements since ammonia is heat-labile. In addition and based on our review of the literature, no previous studies have evaluated the accuracy of POC ammonia testing compared with ammonia concentrations obtained by a standard enzymatic method in a study population consisting of pediatric patients diagnosed with inborn error of metabolism.

Accordingly, the aim of this study was to compare blood ammonia concentrations measured by the new POC device with concentrations measured by standard enzymatic method in patients with known or suspected inborn errors of metabolism. The secondary objective of this study was to evaluate the influence of EDTA coagulant and blood transportation time of less than 30 minutes on ammonia concentration levels. This secondary investigation was considered essential, because general hospitals would likely maintain their POC device in their central laboratory, and the blood specimens would need to be transported from the ward to the laboratory for ammonia concentration analysis by a laboratory technician.

MATERIALS AND METHODS

Patients

This cross sectional study was conducted at the Department of Pediatrics, Siriraj Hospital during the April 2014 to December 2014 study period. Siriraj Hospital is Thailand's largest university-based national tertiary referral center. Included subjects were patients who were either acutely ill with signs and symptoms suggesting hyperammonemia, or patients who had underlying inborn errors of metabolism that required monitoring for blood ammonia at our pediatric genetics clinic. Given that all

studied patients were older than 1 month of age, clinically-significant hyperammonemia in this study was defined as laboratory plasma ammonia >80 µmol/l (Zschocke and Hoffmann, 2011). The protocol for this study was approved by the Siriraj Institutional Review Board (SIRB), Faculty of Medicine Siriraj Hospital, Mahidol University (SIRB approval no. Si427/2013). Written informed consent was obtained from either patients or their legal guardian(s) prior to inclusion in this study.

Blood ammonia measurements

Blood samples were tested for ammonia concentration by both bedside POC ammonia analyzer device (PocketChem BA®; Arkray, Kyoto, Japan) and by enzymatic method, which was performed at the central laboratory of the Department of Clinical Pathology, Siriraj Hospital. The same POC device was used for all analyses. Each blood sample was tested four times for ammonia concentration according to the following steps. Three-ml blood samples were collected from each patient. Twenty microliters of blood was used for immediate measurement of the first blood ammonia concentration with the Pocket Chem BA®, according to manufacturer's protocol. The remaining blood sample was then put into an EDTA tube. EDTA blood was then used for the second measurement of blood ammonia concentration with the PocketChem BA®. The second POC sample was compared to the first sample to determine the possible effect of EDTA on ammonia level. The remaining EDTA blood was then sent on ice to our central laboratory within 30 minutes of blood draw. Any blood specimen arriving at our laboratory later than 30 minutes after blood draw was excluded from our analysis due to the possible release of free ammonia from red blood cells and the deamination of amino acids, which could cause false elevation of ammonia concentrations (Barsotti, 2001). Specimens arriving at our central laboratory within the 30-minutes window were subjected to a third test for blood ammonia concentration using the PocketChem BA®. The third POC sample was compared to the second POC sample to evaluate the effect of transportation time on EDTA blood. The blood remaining after the third measurement was separated using a table-top centrifuge at 960*g* for 10 minutes (Kokusan H-19Alfa; Kokusan, Tokyo, Japan). Ammonia concentration in blood plasma was then measured using the standard enzymatic method. Enzymatic assay was performed using a Cobas 8000 c502 analyzer (Roche Diagnostics, Basel, Switzerland), according to manufacturer's instructions. The measurable range of blood ammonia by PocketChem BA® is between 7 and 286 µmol/l (Goggs *et al*, 2008).

Statistical analysis

SPSS Statistics version 13.0 (SPSS, Chicago, IL) was used for all statistical analyses. Association between blood ammonia concentrations from the POC (first POC sample) and enzymatic methods was assessed by Pearson's correlation coefficient and intraclass correlation coefficient (ICC). Differences in ammonia concentrations between methods was evaluated using Bland-Altman plots. Pearson's correlation coefficient and ICC were used to evaluate ammonia concentration compared between EDTA and non-EDTA samples (first and second POC samples, respectively), and ammonia concentration compared between blood samples measured at bedside and those measured at the laboratory (second and third POC samples, respectively) to evaluate the effect of transportation time less than 30 minutes on ammonia concentrations. Differences in these ammonia concentration comparisons were analyzed using Bland-Altman plots and paired sample *t*-test. Data are presented as number and percentage, mean ± standard deviation, or median and range. A p-value<0.05 was regarded as being statistically significant.

RESULTS

A total of 33 blood samples were collected from 25 patients. Thirteen samples were ex-

cluded, as follows: 10 due to late arrival (>30 minutes after blood draw) at our central laboratory, and 3 due to a 'low' reading on the POC device that could not be included in our statistical analysis. The remaining 20 blood samples from 14 patients yielded 80 measurements (20 samples × 4 measurements = 80 ammonia measurements) for inclusion in our statistical analysis (Tables 1 and 2). Fourteen of 20 (70%) samples were classified as normal by both first POC and enzymatic method, and 4 of 20 (20%) samples were classified as hyperammonemia by both measurements. One of 20 (5%) samples was classified as hyperammonemia by the first POC measurement, but normal by enzymatic method (false-positive). One of 20 (5%) samples was classified as normal by the first POC measurement, but as hyperammonemia by enzymatic method (false-negative). Overall, there was agreement between the two methods in 18 of 20 (90%) samples. Disagreement between methods was only observed in 2 of 20 samples. In only one of the two samples for which there was disagreement between methods, POC testing revealed a normal ammonia result (65 μmol/l), but the enzymatic method revealed hyperammonemia at 84.2 μmol/l, which is slightly above the threshold for hyperammonemia. Using standard enzymatic method for measurement of ammonia concentration as a gold standard, first POC measurements could successfully measure "normal ammonia" levels with 93% agreement. However, in 5 samples with hyperammonemia, first POC measurement could detect hyperammonemia in only 80% (4/5) of cases.

The correlations and differences among the 4 different ammonia concentration measure-

Table 1
Diagnoses of patients whose blood samples were analyzed (total *N* = 20 cases).

Diagnoses	Number (%)
Methylmalonic acidemia	8 (40)
Urea cycle defects	3 (15)
Ornithine transcarbamylase deficiency	2 (10)
Citrullinemia	1 (5)
Isovaleric acidemia	1 (5)
Methylglutaconic aciduria	1 (5)
Multiple carboxylase deficiency	1 (5)
Suspected inborn errors of metabolism	3 (15)

Table 2
Median of ammonia concentrations (μmol/l) from different types of samples.

Types of samples	Median of ammonia concentrations (inter quartile range, μmol/l)
Whole blood (First POC)	41.50 (33.50 - 83.00)
Whole blood with EDTA (Second POC)	45.00 (28.25 - 53.75)
Whole blood with EDTA (Third POC)	49.00 (28.25 - 62.75)
Plasma	55.25 (42.03 - 80.10)

ments of the 20 samples are shown in Table 3. Scatter plot of blood ammonia concentrations from the POC device and the enzymatic method demonstrated a strong positive correlation, with a Pearson's correlation coefficient of 0.95 (p<0.0001) and an intraclass correlation coefficient of 0.93 (95% CI: 0.811-0.974) (Fig 1A). Bland-Altman plot revealed a mean difference of -7.3 μmol/l between ammonia concentration measurements from the POC device and the enzymatic method, with a majority of the bedside POC results being lower than enzymatic method measurement results, and the differences tended to increase when the ammonia values increased (Fig 1B). Ammonia concentrations from bedside POC samples without EDTA (first POC testing) and with EDTA (second POC testing) were positively correlated, without significant difference between methods (p=0.2). Ammonia concentrations from bedside POC samples with EDTA (second POC testing) and POC samples analyzed at the lab (third POC testing) were positively correlated, again – without significant difference between methods (p=0.67).

DISCUSSION

POC testing, including POC glucometer (Ngerncham *et al*, 2012) and POC lactate testing (Martin *et al*, 2013), is widely used in clinical practice due to its speed and reliability. Moreover, given its portability and convenient size, patients and/or their caregivers can monitor these blood tests at home. Although POC testing for blood ammonia concentration was developed several years ago (Huizenga *et al*, 1992), it is still not well-known among clinicians in Thailand. Although plasma measurement for ammonia level by enzymatic method is currently the gold standard, this technique can result in a large number of false-positive results due to a delay in transportation to the laboratory (Broomfield and Grunewald, 2012). The use of POC testing for ammonia concentration could reduce or even eliminate false-positive results. From our

Table 3
Correlations and differences between different four ammonia concentrations of 20 samples.

Comparisons of ammonia levels between samples	ICC (95% CI)	Mean difference (± 1.96 SD) (μmol/l)	Limits of agreement (μmol/l)	Paired sample *t*-tests 95% CI (*p*-value)
1st POC *vs* lab plasma samples	0.93 (0.81-0.97)	-7.33 (± 27.5)	-34.87 – 20.21	Not analyzed
1st POC *vs* 2nd POC samples (without and with EDTA)	0.95 (0.88-0.98)	-4.00 (± 26.4)	-30.44 – 22.44	-10.31 – 2.31 (0.2)
2nd POC *vs* 3rd POC samples (at bedside and the lab)	0.971 (0.93-0.99)	0.95 (± 19.4)	-18.43 – 20.33	-3.67 – 5.57 (0.67)

95% CI, 95% confidence interval; ICC, intraclass correlation coefficient; SD, standard deviations.

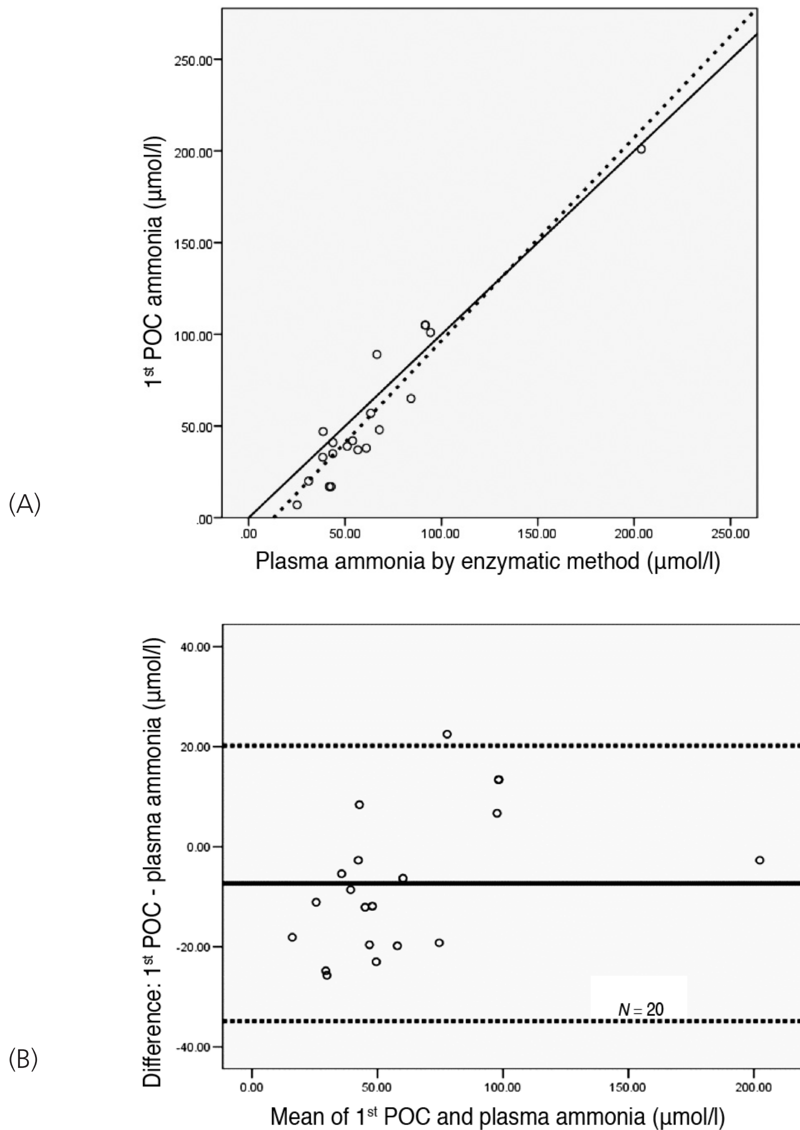

Fig 1– Correlation and difference of ammonia levels obtained from the first POC samples and the enzymatic method. (A) Regression plot shows positive correlation (dotted line). The regression equation is y = 1.1x-14.27 (r = 0.95). The solid line indicates a complete identity line (y = x). (B) Bland-Altman plot of ammonia concentrations obtained from first POC samples and plasma samples. The solid line indicates the mean difference (-7.33 μmol/l) and dotted lines indicate ± 1.96 SD (± 27.5 μmol/l).

study, median ammonia concentrations from different types of samples tended to increase over time and after adding EDTA. These observations may be explained by continued red blood cell metabolism and deamination of glutamine overtime (da Fonseca-Wollheim, 1990), and the addition of non-permeating ions (citrate or EDTA) to whole blood, which results in a shift of

ammonium from the intracellular to the extracellular compartment (da Fonseca-Wollheim, 1995), respectively. However, the majority of ammonia concentrations measured (>90%) from the first POC samples were in agreement with both normal ammonia and hyperammonemia levels from samples evaluated by standard enzymatic method.

All comparisons among the 4 ammonia concentrations of the 20 blood samples demonstrated positive and close correlations, and differences caused by laboratory-related interference, including EDTA and transit time, did not significantly affect ammonia levels. A previous study by Howanitz *et al* (1984) showed that increases in ammonia were significant in whole blood left standing after 1 hour, and that duration had more of an adverse impact on ammonia concentration than temperature relative to how blood specimens are handled. Another study showed that ammonia concentration could increase, even at as early as 10 minutes after collection (da Fonseca-Wollheim, 1990). Our study found that EDTA-containing blood samples and transportation time to a laboratory of less than 30 minutes do not cause significant changes in ammonia concentrations. As indicated by Bland-Altman plot in our analysis, POC ammonia concentrations demonstrated a negative constant bias, given that the majority of the first POC ammonia concentrations were lower than the enzymatic method ammonia concentrations by -7.3 μmol/l. This limitation of POC could result in an underestimation of blood ammonia or a false-negative result, as we observed in one patient that was mentioned earlier. Therefore, clinicians who use this POC device should be cautious for false-negative results when the obtained POC ammonia concentrations are generally consistently lower than ammonia concentrations obtained by enzymatic method.

Due to its upper limit of detection of 286 μmol/l, ammonia measurement by POC device will only allow detection of modest levels of hyperammonemia. In general, patients with inborn errors of metabolism, especially during acute hyperammonemic crises, could have ammonia concentrations that exceed 300 μmol/l (Haberle, 2011). As such, this device may not be suitable for monitoring patients with severe hyperammonemia. Rather, the POC ammonia analyzer may be better suited to screening for hyperammonemia in cases where there is clinical suspicion for hyperammonemia. Cases with increased ammonia levels by POC device should be promptly referred to a metabolic specialist.

This study has some mentionable limitations. First, our study population was rather small, and the number of subjects with hyperammonemia (plasma ammonia >80 μmol/l) was even smaller. This limitation may have limited our ability to identify all significant associations and differences between the two studied methods. Moreover, due to a limited number of samples with hyperammonemia, the true differences between first POC and standard enzymatic method remain unclear.

In conclusion, we found 90% agreement between POC ammonia concentration measurement and standard enzymatic measurement in this study. Therefore, POC ammonia testing may be a suitable method for hyperammonemia screening in general hospitals where blood ammonia concentration measurement by standard enzymatic method is not available. Moreover, the advantage of POC ammonia testing is the cost of test could be cheaper than the enzymatic method when amount of specimens is modest. This diagnostic tool would facilitate simple and fast detection and timely referral in patients with hyperammonemia.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the patients and families that participated in this study, and Dr Chulaluk Komoltri for assistance with statistical analysis. This study was supported by

a grant from the Siriraj Research Development Fund (R015731067).

CONFLICTS OF INTEREST

The authors hereby declare no personal or professional conflicts of interest regarding any aspect of this study.

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