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Evaluation of Analyzer and Measurement Conditions of Blood Ammonia

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혈중 암모니아의 측정조건과 분석기기의 평가

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Ammonia is very toxic, and causes neuronal damage via excitotoxicity, oxidative stress, and inflammation. Because the liver is the primary organ for ammonia metabolism, compromised liver function can result from inborn errors of metabolism. Measurement of blood ammonia has some limitations. Recently, several laboratories examined possible concurrent increases in plasma ammonia. However, the collection, handling, storage, and analysis of blood samples are all potential sources of error. For evaluation of rapidity and reliability of measurement of blood ammonia, the DRI-CHEM 100 (Fuji Film Co., Japan) and COBAS 8000 (Roche Diagnostic Ltd., Switzerland) analyzer were used for analysis of ammonia level values. The results of this study detected a high correlation between analyzer. Therefore, one-step measurement was suitable for ammonia analysis. After sampling of the ammonia in the time slot for measurement an increase to 46.5, 57.4, and 79.0 (μg/dL) was observed at 30, 90, and 180 minutes. In addition, specific capacity of the ammonia, 7, 10, and 13 (μL), was measured as 39, 46, and 43 (μg/dL), respectively, and the FDC-100 analyzer was more effective in 10 μL ($p < 0.001$). In conclusion, the evaluated analysis may offer useful information for clinical application.

Keywords: Ammonia, Evaluation, One-step

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Introduction

Ammonia is recognized as a toxin central to complications of liver failure. Ammonia is believed to play a key role in the development of hepatic encephalopathy (HE) with increased formation of glutamine playing a central role. Cirrhosis represents the final common histological pathway for a wide variety of chronic liver diseases. The blood ammonia levels of cirrhotic patients are usually higher than those of normal people [1,2]. Ammonia levels correlated with the severity of hepatic encephalopathy. Greater the ammonia level, severe is the grade of hepatic encephalopathy [3]. Generally, ammonia is produced by glutamine metabolism in the small bowel and bacterial flora in the large intestine. The urea cycle is the major pathway of nitrogen metabolism in the human body. Excess nitrogen, in the form of ammonia, is converted via this cycle to urea and excreted through the kidneys. In humans, the cycle entails five key enzymes, including carba-

moyl-phosphate synthetase I (CPS1), ornithine transcarbamylase (OTC), argininosuccinate synthetase, argininosuccinate lyase and arginase; while an additional enzyme named N-acetylglutamate synthase provides CPS1 with its essential cofactor [4].

Hyperammonemia is thought to be central in the pathophysiology of hepatic encephalopathy in patients suffering from liver failure. Plasma ammonia has been used in emergency departments to assess whether or not generalized convulsion attacks exist in patients who are suspected of having convulsions. However, there are few reports that have assessed the relationship between generalized convulsions and hyperammonemia. Plasma ammonia values rise during generalized convulsion. Measurement of plasma ammonia is clinically highly significant as an independent finding during the diagnosis of generalized convulsion [5].

Most analysis devices are increasingly derived to perform repetitive acting to human operators, accuracy, speed, convenience, and cost for advantages. Recently, a device for measuring human breath ammonia was developed based on a single rapidly use, disposable, inkjet printed ammonia sensor fabricated using polyaniline nanoparticles. The device was optimized for sampling ammonia in human breath samples by addressing issues such as variations in breath sample volume, flow rate, sources of oral ammonia, temperature and humidity [6]. Excellent correlations were conducted between breath ammonia and blood urea nitrogen (BUN), which it is the possibility of breath ammonia systems as a monitoring kidney dysfunction and treatment.

Death is likely to result in very extensive biochemical changes in all body tissues due to lack of circulating oxygen, altered enzymatic reactions, cellular degradation, and cessation of anabolic production of metabolites. These biochemical changes may provide chemical markers for helping to more accurately determine the time since death (post-mortem interval), which is the larger than the increase seen in post-mortem rat blood and *in vitro* experiments [7]. Increased arterial ammonia levels are associated with high mortality in patients with acute liver failure (ALF), which is the elevated arterial ammonia levels indicate a poor prognosis in acute liver injury [8].

The most commonly used method in clinical labs is an enzymatic kinetic assay in which ammonia reacts with α-ketoglutarate and nicotinamide adenine dinucleotide phosphate (reduced form; NADPH) to form glutamate and $NADP^+$. The amount of ammonia is equivalent to the amount of NADPH oxidized, which can be measured photometrically.

The hemolysis samples were found to have a significantly lower impact on the measurement of plasma ammonia, and then sample separation and storage times were have to a lower stabilized the plasma [9]. Also, the plasma ammonia concentration were elevated in osmotic shock, preparing a plasma by removing plasma from a whole blood, spinning, washing the cells with saline, and freezing overnight.

The frozen hemolysate is thawed the next day, serially diluted to different levels with saline, and spiked into plasma sample aliquots to produce different degrees of hemolysis [10]. The measurement of plasma ammonia is an important screening investigation for many inborn errors of metabolism, but mild to moderate hyperammonaemia can occur as a non-specific finding in sick children, particularly neonates [11]. We are showed that investigation of the DRI-CHEM 100 (Fuji Film Co., Saitama, Japan) and COBAS 8000 (Roche Diagnostic Ltd., Rotkreuz, Switzerland) analyzer, a new technically whole blood ammonia analyzer compared to general serum ammonia analyzer for clinical use by determining machine precision, linearity, repeatability and accuracy and optimal condition of measurement according to amount and time after sample collection.

Materials and Methods

1. Participants

The study was carried out in an open population of healthy adults (n=72) aged 20~25 years in Namseoul university. They were participated by means of announcements made by the voluntary purpose. The study was approved by institutional review board at Namseoul university (NSU-140428-2). Written informed consent was obtained and signed by all participants prior to the blood collection.

Both females (n=60) and males (n=12) were considered healthy blood donors as determined by the analysis of a

written questionnaire (previous disease or acquired disease). Individuals were excluded from the study with diagnosis of hemorrage disease. We additionally excluded subjects with previous or recent diagnosis of severe diseases, chronic hepatic disease.

2. Blood analysis

All participants were measured in drawn blood samples with the instrument DRI-CHEM 100 (Fuji Film Co.), which used a 10 μL of whole blood obtained from an EDTA bottle and the plasma sample were obtained by centrifugation. then the measurement the ammonia using the instrument COBAS 8000 (Roche Diagnostic Ltd.) analyzer. For COBAS 8000 analyzer, the coefficients of variation were determined by repeated measurement of 2 control solutions. Linearity was investigated by testing serial dilutions of a stock solution. These tests were subsequently repeated for individuals who had significant clinical alterations. using standard laboratory analysis techniques.

For accuracy, samples from clinical cases were used to compare the results on the DRI-CHEM 100 and COBAS 8000 analyzer reference method. Patients were consecutively enrolled if blood ammonia was assayed and samples could be analyzed shortly after collection. Classification of results (as normal or high, using 100 μmol/L as a cutoff value) and intraclass correlation coefficients were used to compare the methods. Stability of samples and test strips also was assessed room at temperature.
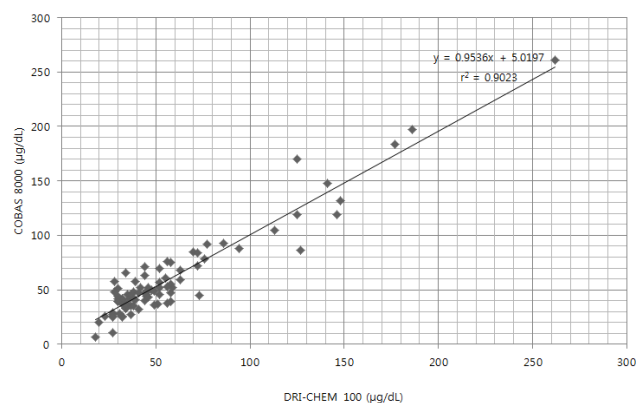
3. Statistical analysis

To evaluate the annual correlation distribution of each performed Chi-square test, level of significance was set the $p<0.05$ which is statistical significance level and spearman's correlation analysis was incorporated for the assessment of the extent of correlation after relevant variables were compensated. The level of statistical significance was defined as having a p-value of less than 0.05 or 0.01. Data were analyzed using PASW version 17.0 (SPSS Inc., Chicago, IL, USA). The correlation was analyzed using pearson correlation coefficients.

Results

1. Comparative correlation of measured ammonia levels between DRI−CHEM 100 and COBAS 8000 analyzer

The results of correlation between the ammonia measued by the DRI-CHEM 100 and COBAS 8000 analyzer analyzer was resulted from the same blood samples (n=72). The value of r is 0.9499. This is a strong positive correlation, which means that high X variable scores go with high Y variable scores. The value of r^2, the coefficient of determination, is 0.9023 (Fig. 1).

Especially DRI-CHEM 100 analyzer using a immediately whole blood without centrifugation for plasma. Whereas, The pre-treatment was required to separate the plasma for analysis is a COBAS 8000 analyzer. Pre-treatment time was takes an average of 21.25 minutes. In addition, calibration was performed daily for analysis. Showed a significant difference in the two devices is a significant correlation between the two devices. but, out of normal range ($>$100 μg/dL) ammonia value was not significantly. The results of

Fig. 1. Correlation of scatter plot diagram with measured ammonia value by DRI−CHEM 100 and COBAS 8000 analyzer.

Table 1. Correlation of ammonia value measured by DRI−CHEM 100 and COBAS 8000 analyzer (r[p])

Variable	DRI−CHEM 100	COBAS 8000
DRI−CHEM 100	1	
COBAS 8000	0.950* (0.000)	1
Mean±SEM	56.86±41.09	59.24±41.25

*$p<0.01$; Average of time interval for analysis in two apparatus was 21.25±10.28 min.
Abbreviation: SEM, standard error of mean.

correlation to measured ammonia value by DRI-CHEM 100 and COBAS 8000 analyzer ($p<0.000$) (Table 1).

2. Ammonia levels compared to the time interval after blood collection

Ammonia was measured by difference hours after blood collection. To evaluate the changes in ammonia levels result from the sample (whole blood) after blood collection time. As measured by DRI-CHEM 100 result, there was a tendency to increase the ammonia concentration with the passage of 30, 90, 180 minutes after the blood collection (46.5, 57.4, 79.0 μg/dL) (Fig. 2).

The correlation change of each time ammonia analysis after blood collection (Table 2). It was showed the highly correlation in 90, 180 minutes compared to 30 minutes to between the three groups.

3. Ammonia comparison value according to the different amount

The results of the value corresponding to the amount of whole blood to be used for the analysis of ammonia. Increased

levels of ammonia relative to the amount more 10 μL than 7 μL and 9 μL (Fig. 3).

The results of investigating the correlation between the three groups, The significantly correlated in 10 μL and 13 μL than 7 μL. This is less than 10 μL volume seems to indicate an error in the measurement (Table 3).

Discussion

Acute on chronic liver failure (AoCLF) is associated with a high mortality rate. Plasma exchange (PE) is useful to bridge AoCLF patients to liver transplantation. In this time, ammonia may be important in the pathogenesis of the AoCLF and PE may represent a reliable hepatic support device for AoCLF [12].

Ammonia in a plasma sample can be falsely increased by contamination by atmospheric ammonia, by smoking, or by prolonged stasis during venipuncture reference. If the sample is not centrifuged and analyzed promptly, ammonia is formed by the continuous deamination of amino acids. The concentration increases by 20% in the first 1 hour and by up to
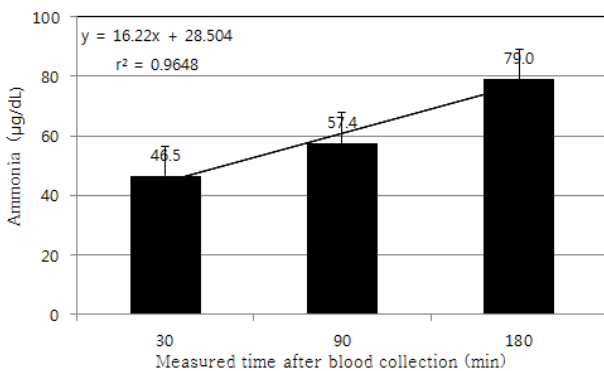

Fig. 2. Histogram of the measured values according to the analysis time after blood collection.

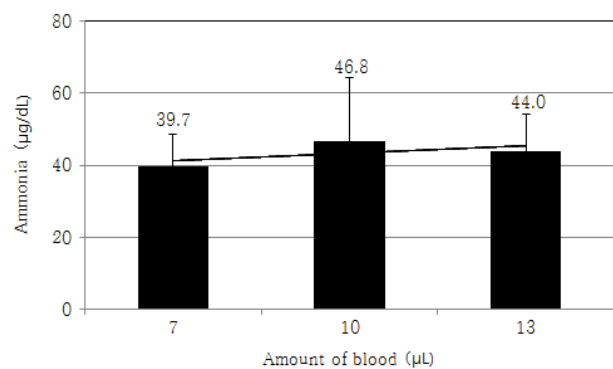Fig. 3. Changes of ammonia levels in accordance with the difference amount of used blood for analysis.

Table 2. Correlation of ammonia value with measured interval time after blood collection by DRI-CHEM 100 analyzer (r[p])

Variable	30 min	90 min	180 min
30 min	1		
90 min	0.867* (0.000)	1	
180 min	0.571* (0.000)	0.420* (0.006)	1
Mean±SEM	46.51±10.07	57.37±10.64	78.95±10.32

*$p<0.01$.
Abbreviation: SEM, standard error of mean.

Table 3. Correlation of ammonia value with blood amount by DRI-CHEM 100 analyzer (r[p])

Variable (μL)	7	10	13
7	1		
10	0.442* (0.035)	1	
13	0.833** (0.000)	0.030 (0.894)	1
Mean±SEM	39.70±8.78	46.78±17.47	43.96±10.29

*$p<0.05$, **$p<0.01$.
Abbreviation: SEM, standard error of mean.

100% by 2 hrs. Plasma samples for ammonia testing should be placed in ice water immediately and transported for analysis as soon as possible. Increased ammonia in a patient with an AMS (altered mental status) change is a critical laboratory finding that should be addressed immediately [13,14].

Plasma ammonia concentration was correlated with the mean rate of remifentanil and creatinine clearance [15]. Ammonia production is not directly related to intrafollicular female sex hormones concentrations [16]. Hyperammonemia was significantly correlation between venous ammonia level and arterial pH on emergency room arrived or out-of-hospital cardiac arrest patients. The measurement of ammonia was found to provide valuable information regarding neurological outcome [17].

Among the blood measurements on emergency department arrival, blood ammonia (>96 mg/dL) was the predictive biomarker of poor neurologic outcome diagnosis. Thus, higher blood ammonia level was associated with neurologic outcome in OHCA (out-of-hospital cardiac arrest) patients [18].

The stability of a wide range of plasma analytes in whole blood samples stored for several days is usually believed. However, whole blood were unconditionally necessary for blood ammonia analysis. In the aspect to laboratory analysis, the accuracy of blood ammonia assays rely on the specimen collection, treatment and the analytical method. Thus, New methods followed in ammonia (point of care test) and noninvasive techniques (quantification of ammonium in the breath) and the rapidly advanced. To identify factors that affect plasma ammonia levels or metabolism to its analysis, the various analysis of plasma ammonia level and increased the severity of hepatic encephalopathy because of individual differences in ammonia metabolism and differences in the accuracy of analytical methods [19].

These results are relevant for planning blood-based (whole blood or plsma) laboratories. Depending upon the analytes to be measured, blood pretreatment methods could be greatly simplified and, hence, the costs vastly reduced [20]. The plasma ammonia analyzer has acceptable precision, adequate linearity, and satisfactory agreement with a reference method, but negative constant and proportional biases.

These analyzer may be suitable for clinical use in patients suspected of having hepatic encephalopathy, benefit a using the lower reference and decrease false negative results. For accuracy, Time of analysis was <90 min and amount of whole blood was >10 μL for ammonia analysis with DRI-CHEM 100 analyzer. Accurate measurement of blood ammonia is useful in diagnosing inherited disorders of urea metabolism and diagnosis of hepatic encephalopathy. The findings may offer useful information for clinical management.

요 약

암모니아는 매우 독성이 있으며, 흥분 독성, 산화 스트레스, 염증을 통해 신경 세포의 손상을 유발한다. 간이 암모니아 대사를 위한 일차 기관이라는 사실에 근거하여 선천성 대사 오류의 원인이 된다. 혈중 암모니아 판정은 측정값의 일부 범위에서 결정하게 되는데 최근 진단분야에서 혈중 암모니아를 가능한 동시다발적으로 측정하게 되었다. 그러나 혈액검체의 수집, 처리, 저장 및 분석은 오류의 모든 잠재적인 요인이다. 신속하고 신뢰할 수 있는 혈중 암모니아 측정의 평가를 위해 DRI-CHEM 100 (Fuji Film Co., Japan) 및 COBAS 8000 (Roche Diagnostic Ltd., Switzerland) 분석기를 이용해 비교평가 분석하였고 높은 상관성을 얻었으며 one-step 방법은 암모니아 분석에 적합하였다. 암모니아의 채혈 후 시간대별 측정에서는 30, 90, 180분에 각각 46.5, 57.4, 79.0 (μg/dL)로 상승하는 경향을 보였다. 또한 암모니아의 용량별 측정에서는 7, 10, 13 (μL)에 각각 39, 46, 43 (μg/dL)으로 나타났으며 10 μL에서 유의성을 보였다($p<0.001$). 결론적으로 위 평가 분석은 임상적용에서 유용한 정보를 제공 할 수 있을 것이다.

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