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 transcar-
bamylase (OTC), argininosuccinate synthetase, argininosuc-
cinate 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 patho-
physiology of hepatic encephalopathy in patients suffering
from liver failure. Plasma ammonia has been used in emer-
gency 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]. In-
creased 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
\( \alpha \)-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 hemorrhage 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 measured 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

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\begin{array}{|c|c|c|}
\hline
\text{Variable} & \text{DRI–CHEM 100} & \text{COBAS 8000} \\
\hline
\text{DRI–CHEM 100} & 1 & \text{0.950* (0.000)} \\
\text{COBAS 8000} & \text{0.950* (0.000)} & 1 \\
\text{Mean±SEM} & 56.86±41.09 & 59.24±41.25 \\
\hline
\end{array}
\]

* \( p < 0.01 \): 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 (ρ<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

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

| 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 |

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

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

| 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 |

*ρ<0.05, **ρ<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 correlated 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 plasma) 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.

요약

암모니아는 매우 독성이 있으며, 흥분 독성, 산화 스트레스, 염증을 통해 신경 세포의 손상을 유발한다. 간이 암모니아 대사를 위한 임차 기관이라는 사실에 근거하여 선천성 대사 오류의 원인이 된다. 혈중 암모니아 측정은 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 이용에 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 보었다. 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 높 평가 분석은 암양적응에서 유용한 정보를 제공할 수 있을 것이다.

Acknowledgements: None
Funding: None
Conflict of interest: None

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