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RESEARCH ARTICLE

The influence of transport condition and processing time on plasma ammonia results

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Objective: Ammonia is extremely unstable in blood specimens and has special requirements during transport, processing and storage. The aim of our study was to determine the stability of ammonia in EDTA K3 blood samples and to establish a protocol for sample handling. **Methods**: In this study, 36 healthy subjects and 47 inpatients diagnosed with type 2 diabetes mellitus were enrolled. Two peripheral blood samples were collected from healthy volunteers (Sample A1 and A2) and one peripheral blood sample was collected from the inpatients diagnosed with type 2 diabetes mellitus (Sample B). Sample A1 and B were transported in ice bath within 15 minutes of blood collection, centrifuged immediately and processed. The sample was re-centrifuged after 15 minutes and a second ammonia result was obtained. Sample A2 was transported at room temperature and stored between 2 and 4 hours, centrifuged and plasma ammonia measurement was performed. The sample was re-spun after 15 minutes and a fourth ammonia result was obtained. **Results**: In our study, in healthy group the difference between sample A2 and set point value (on ice, 15 minutes) is 25.08 µg/dl, showing an increase of 55.29%. After another 15 minutes, an increase of 82.02% was observed compared with the standard value. In diabetes mellitus group, after 30 minutes of blood collection, an increase of 11% over the set point value was observed. **Conclusions**: The blood specimen should be transported on ice to the laboratory and analyzed within 15 minutes of blood collection due to plasma ammonia spontaneously increase.

Keywords: ammonia, diabetes mellitus, temperature

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Introduction

Ammonia is the metabolic waste product of amino acid catabolism [1]. The largest quantity is produced by protein digestion, in the presence of intestinal bacteria [1,2]. In the renal proximal tube, ammonia results from glutamine metabolism and is excreted by urine or released into the vascular system [3]. Nowadays, the classic model of ammonia transport with "NH_4^+ trapping" is being replaced by a model in which specific proteins are able to transport NH_4^+ across plasma membranes. This mechanism is important to renal ammonia excretion [3,4]. In case of increased physical exercise or low glucose level, the skeletal muscle can produce ammonia through amino acid catabolism [1].

Ammonia can produce intracellular acidification or alkalization depending on pH, concentration or rate of NH_3 versus NH_4^+ transport and can generate alterations in cellular metabolism, cell function and signaling pathways, impacts activities of enzyme or protein phosphorylation [5]. Because ammonia is neurotoxic itself, it is promptly converted to urea by the urea cycle. The urea cycle is located in periportal hepatocytes and it represents the main pathway to detoxify ammonia [6].

In liver diseases such as hepatitis and cirrhosis or urea cycle inborn disorders ammonia level increases [1,7]. Clinical symptoms in hyperammonemia are not specific and are related to the underlying condition. Most symptoms are neurological such as mood and personality alteration, disorientation, hallucinations, ataxia, seizures, brain edema, coma [8]. To date, the most widely used treatments are antibiotics and lactulose, designed to decrease intestinal ammonia production or its absorption, but followed by side effects [9,10]. Untreated, hyperammonemia could cause irreversible brain damage [11].

Ammonia measurement is essential for many metabolic disorders but ammonia is extremely unstable in blood specimens. The specimen must be transported on ice and processed within 15 minutes after collection. Plasma ammonia levels increase in vitro due to amino acid degradation and red blood cells lysis [12].

The aim of our study was to determine how the transport temperature and centrifugation time impacts the stability of ammonia in tripotassium ethylenediaminetetraacetic acid (EDTA K3) blood samples and to establish and optimize an appropriate protocol and rules that must be applied in sample acceptance in Emergency Clinical County Hospital of Targu-Mures. Furthermore, another objective was to analyze plasma ammonia levels in diabetic mellitus inpatients and compare the results with the healthy subjects.

Methods

In this cross-sectional study, 36 healthy subjects (Group A) and 47 inpatients diagnosed with type 2 diabetes mellitus (Group B) from Emergency Clinical County Hospital of Targu-Mures were enrolled. The study was approved by the

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Ethics Committee of the Emergency Clinical County Hospital of Targu-Mures and all patients signed an informed consent form.

One peripheral blood sample was collected from healthy volunteers using 3.4 mL EDTA K3 tubes (Sample A1). Blood was collected by standard venipuncture techniques using a 23 gauge sterile needle. The venipuncture site was disinfected with an alcoholic pad. The sample was placed in an ice bath, transported to the laboratory within 15 minutes after blood collection, centrifuged for 10 minutes at 1780 RCF, and the first measurement was performed. The sample was stored at room temperature (RT) for 15 minutes and then was re-centrifuged under the same conditions and a second measurement was performed. A second blood sample was collected only from the healthy volunteers using 3.4 mL EDTA K3 tubes, transported at room temperature, stored between 2 and 4 hours, spun for 10 minutes at 1780 RCF and processed (Sample A2). The blood sample was centrifuged again after 15 minutes using the same protocol and another ammonia result was obtained.

From the 47 inpatients diagnosed with type 2 diabetes mellitus one peripheral blood sample was collected (Sample B). The sample was placed in an ice bath, transported to the laboratory within 15 minutes of blood collection, centrifuged for 10 minutes at 1780 RCF, and processed. The sample was stored at RT for 15 minutes, then was re-centrifuged and a second measurement was performed (Figure 1).

Centrifugation of blood samples was performed with Hettich Rotofix 32 centrifuge. Plasma ammonia level was measured on the Architect c4000 analyzer (Abbott, IL, USA) using an enzymatic assay based on the glutamate dehydrogenase (GLDH) method. The instrument was calibrated with a one-level calibrator (Ammonia Ultra, Abbott Laboratories Inc., IL, USA) and quality control was performed each day with Control Architect Sentinel Ammonia. The reference range recommended by the manufacturer was 31 to 123 µg/dl (18 to 72 µmol/L) and the limit of detection was 8 µg/dl (4.70 µmol/L).

The statistical analysis was performed using the MedCalc software program and significance threshold was set at $p<0.05$.

Results

In Group A out of the total of 36 volunteers, 23 were females and 13 males. The average age for healthy subjects was 56 years, with ages between 21 and 82 years old.

The mean of set point from sample A1 measurements was 46 +/- 10.66 µg/dl with a normal distribution ($p = 0.053$). An ANOVA repeated measures test was performed to compare results from samples transported and stored in different conditions. Plasma ammonia concentration in Sample A1, after 30 minutes of blood collection was 53 +/- 18 µg/dl. The Sample A2 ammonia concentration mean was 71 +/-21 µg/dl after 2-4 hours at RT. After another 15 minutes at RT mean value was 83 +/-23 µg/dl. Results are shown in Figure 2.

In Group B out of the total of 47 diabetic patients (medically controlled), 28 were females and 19 males. Plasma ammonia concentration in Group B from the Sample B1 was 45.31 +/- 20.3 µg/dl. After 30 minutes of blood collection, plasma ammonia concentration mean value was 50.31 +/-20.3. Results for group B are shown in Figure 3.

Discussions

A gradual increase in plasma ammonia results was observed. Ammonia level continued to rise in vitro due to amino acid degradation. Therefore, blood specimens must be properly transported and analyzed as soon as possible [12,13]. The current specimen handling protocol was based upon numerous studies that demonstrated a real increase in plasma ammonia concentration [14].

RT storage

In Group A after 30 minutes of blood collection, an increase of 16% was observed when comparing with the setpoint value ($p<0.01$). In a study performed by Da Fonseca et al., stability of 15 minutes in dipotassium ethylenediaminetetraacetic acid (EDTA K2) sample spun at RT was observed [11,14]. However, in a study published by Favresse et al. ammonia was stable 30 minutes in 20 EDTA K2 samples analyzed at RT [12].

4°C storage

Goldstein et al. declared stability of 13 to 15 hours in EDTA K2 plasma samples kept at 4°C [16]. In a retrospective study, Hashim and Cuthbert did not found a signifi-

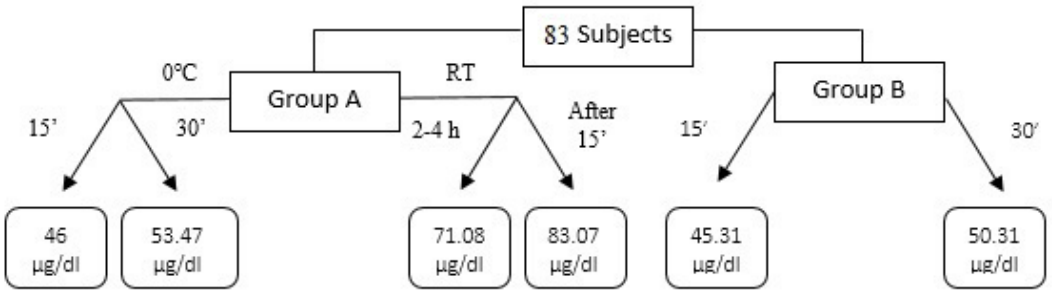

Fig. 1. Classification of specimens from group A and group B (plasma ammonia concentration for sample A1 and B at 15, respectively 30 minutes and for sample A2 at RT, stored 2-4 hours and after other 15 minutes).

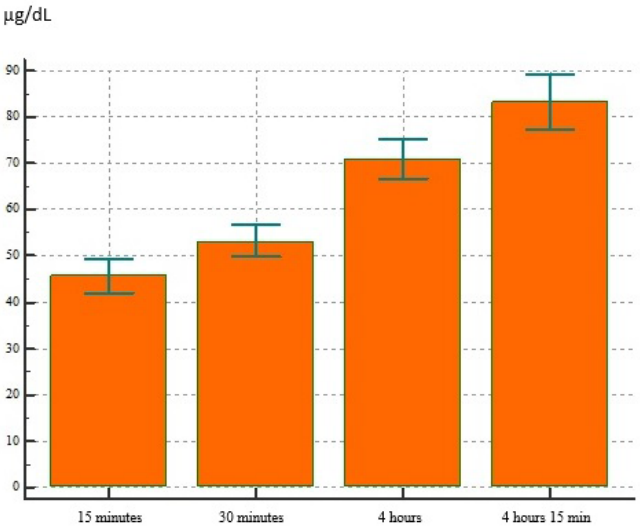

Fig. 2. Changes in plasma ammonia concentration in group A. This chart highlights the increase in plasma ammonia concentration after 30 minutes compared with set point value in healthy subjects.

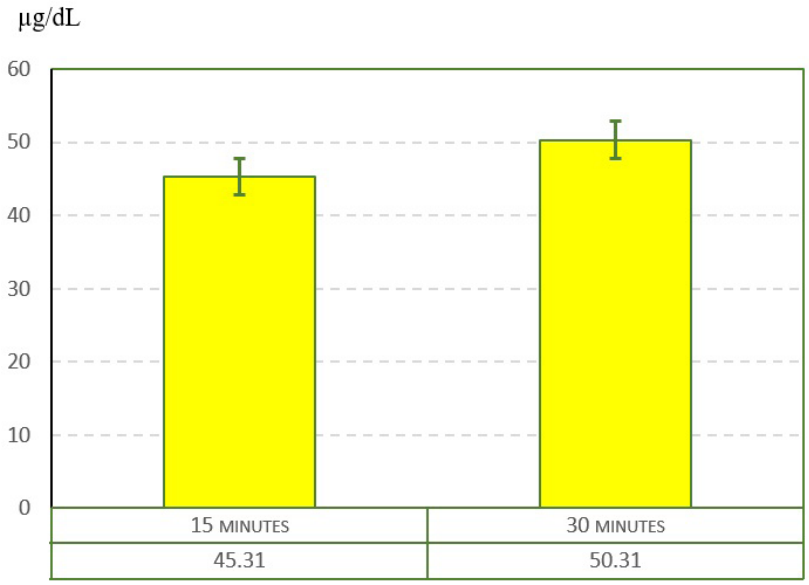

Fig. 3. Changes in plasma ammonia concentration in group B. This chart highlights the increase in plasma ammonia concentration after 30 minutes compared with set point values in diabetic patients.

cant relationship between plasma ammonia levels and pre-analytical time up to 30 minutes in 1800 EDTA samples transported on ice and centrifuged at 4°C [17].

In a study performed by Jack Hester 30 minutes of plasma, ammonia stability was found on 44 EDTA K3 blood samples kept on ice or RT [10].

In our study the difference between plasma ammonia concentrations measured after 2-4 hours at room temperature (Sample A2) and setpoint value (on ice, 15 minutes) was 25.08 µg/dl, showing an increase of 55.29% ($p<0.01$). After another 15 minutes, an increase of 82.02% was observed compared with the standard value ($p<0.01$).

The values obtained from healthy subjects were compared with the reference value from the package insert. The mean value in our group was 46 +/-10.66 µg/dl . The reference value reported by the manufacturer was 31 to 123 µg/dl. A major difference between the upper limit of the reference values and plasma ammonia values obtained in our study was observed.

Diabetic patients

In Group B, after 30 minutes from blood collection, an increase of 11% ($p<0.01$) over the set point value was observed. In our study, we did not find a statistically significant difference between Group A and Group B, but we observed that there was better stability of ammonia in patients with diabetes. A study published by Gunanithi. K et al. showed that ammonia levels are much higher in uncontrolled type 2 diabetes patients compared to healthy people [18].

Controversy may appear in interpreting results from the studies due to different sample types, the number of

subjects included in the study, time to measurement, and transport conditions. In a study performed by Goldstein et al. ammonia was stable 13-15 hours at 4°C in EDTA K3 blood sample, but in a study published by Dukic and Simundic, 1-hour stability in lithium heparin plasma sample stored at 4°C was observed [16, 17].

Pneumatic transport

In a study performed by Bismut et al. in a large university hospital they observed that despite the pneumatic transport, the time interval for sample arrival to the laboratory were longer than those recommended by manufacturer. However, ammonia concentration results were not influenced by the pneumatic transport, they showed a maximum of 105 min before centrifugation and 1 h after centrifugation stability in ammonia sample [20].

We consider that it is important to establish a protocol and to determine the reference range based upon local particular characteristics of the Targu Mures population. In this case, according to Clinical & Laboratory Standards Institute (CLSI), it is necessary to increase healthy group size to 162 and perform plasma ammonia measurement. Thus, a reference interval adapted to the population of Targu-Mureș can be achieved.

Limitations of the study: the small sample size.

Conclusions

In conclusion, according to our findings, we recommend that the ammonia EDTA K3 sample to be placed in an ice bath, transported to the laboratory within 15 minutes after blood collection, and processed immediately. In addition, better ammonia plasma stability in diabetic patients was observed compared to healthy subjects.

Authors' contribution

KC – Conceptualization, Data curation, Investigation, Methodology, Validation, Writing - original draft, Writing - review & editing

OO – Conceptualization, Formal Analysis, Investigation, Methodology, Writing - review & editing

MD – Methodology, Supervision, Validation, Writing - original draft, Writing - review & editing

Conflict of interest

None to declare.

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