Effect of Preanalytical Techniques and Variables on Plasma Ammonia Estimation

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ملخص

إن ازدياد او انخفاض تركيز الأمونيا في بلازما الدم يعود إلى التعامل الخاطيء مع عينات الدم. ومن بين العوامل الكثيرة التي تؤثر على تركيز الأمونيا في بلازما الدم عملية تجميع عينات الدم، أنواع مضادات تجلط الدم للحصول على البلازما، قوة الطرد المركزي المستخدمة لفصل البلازما عن الدم بالإضافة إلى الجو ودرجة الحرارة التي تخزن فيها عينات الدم.

تم التركيز في هذه الدراسة على تأثير قوة الطرد المركزي المستخدمة لفصل البلازما وعلى أنواع مضادات تجلط الدم، وأيضاً على كمية الدم غير المتجلط في أنابيب التجميع. تم سحب عينات من الدم الغير متجلط من من عشرة متطوعين ذكور استخدموا في هذا الدراسة.

أظهرت النتائج أن استخدام عينات غير متجلطة وغيره متكسرة، وأيضاً استخدام قوة طرد مركزي محدودة (١٥٠٠ - لمدة ١٥ دقيقة) كانت من الأهمية في تجنب حدوث ازدياد في تقدير الأمونيا. وأظهرت الدراسة تفضيل استخدام مادة مانعة التجلط (EDTA) على غيرها من موانع التجلط وأن كمية الدم في انابيب التجميع لا تؤثران على تركيز الأمونيا في هذه العينات. وقد خرجت هذه الدراسة باستنتاج عام أن الطرق

المثالية التي بحثت في هذه الدراسة في التعامل مع عينات الدم من تجميعها وتخزينها من أجل قياس الأمونيا في هذه العينات، يجب أن تتبع بحذافيرها.

ABSTRACT

Ammonia concentration may increase or decrease due to the mishandling of blood specimens. Among the factors that affect ammonia concentration, blood collection, type of anticoagulant, centrifugation forces, atmosphere and storage temperature. We focused on the effect of centrifugation forces, type of anticoagulant and the complete filling of the blood collection tube. Ten male healthy volunteers, each with replicate specimens were used in this study. Results show that the use of nonhemolysed, non clotted specimen and prompt centrifugation (1500 X g; 15 minutes) apparently were important in avoiding such increase in ammonia concentration. It was also found that EDTA - Plasma is preferred over the other anticoagulated specimen for ammonia determination. Complete filling of the specimen tube was found to have no significant effect on ammonia concentration. We concluded that the procedure used for collection and storage of specimens for ammonia determination should be standardized and strictly observed.

Introduction:

Ammonia test is an important indicator for the diagnosis and follow up of several hepatic and renal disorders (1,2). the effect of various conditions on ammonia determination was depated by Balistreri et al 1, Varely (3) and Gerron et al. (4)

Reference intervals vary with age and sex of the subject and type of the sample (5,6,7). diaz et al. (7) found that blood

ammonia concentration decreased with age (1 year to 14 years) and the concentration was higher in men than in women. There was a significant difference between concentration of ammonia in heparinized plasma (30 μmole/L) and serum specimen (44 μmole/L)(8).

To fill the specimen tube completely as recommended by Doumas et al. (9) requires a minimum of 6 to 8ml of blood and this is often difficult in the case of critically ill babies. It is found that technical error such as unproper mixing of the anticoagulant with blood sample leads to a clotted sample, also in a busy emergency laboratory, the blood sample may not consequently be adequately centrifuged. These factors are equally important and might affect ammonia concentration.

The objective of this study is to investigate some of these factors that affect the ammonia concentration and to minimise the artificial fluctuations of ammonia concentration in blood samples.

Materials and Methods:

Specimen: venous blood samples from healthy male volunteers (25 years old $\pm$ 1.0) were collected into three types of collection tubes; potassium citrate - EDTA; Lithium heparin and plain tubes were obtained from Greiner, Labtechnik.

Three groups of samples were collected from each volunteer through out the study and were immediately put on ice. Plasma and serum were separated and ammonia concentration was measured with a discrete analyser the ACA, with ammonia reagent packs (both obtained from dupont Instruments, Wilmington, DE 19898).

Ammonia Standard and Control:

40 and 80 µmole/L controls of ammonium chloride solutions were prepared from ammonium chloride solution (400 µmole/L) as recommended by Howanitz et al. (8). This solution was supplied by the Dupont ammonium calibrators and used to determine the instrument day - to - day percision.

Procedure of Variable Factors Investigation:

The following variables were investigated in this study:

1 - The effect of filling the specimen in collection tubes. From paired collection tubes (n = 10), one was half filled and the other was fully filled with the same sample.

2 - The effect of centrifugation on the specimen. Using the second group of paired samples, one of each pair was centrifuged for five minutes at low speed (500 × g) and the other was centrifuged for 15 minutes at a high speed (1500 × g). Five paired samples were used in this study.

3 - The effect of clotting. From the third group of paired samples, one of each pair was properly mixed with the anticoagulant (potassium citrate EDTA) while the other was not mixed, resulting in clotted samples. Five paired sample were examined.

Results and Discution:

The within-day CV for analytical variation was 2.5% and 4.3% for the 40 and 80 µmole/L controls. Since there is a difference in the concentration of ammonia between male and female (Diaz et al. (7). All volunteers who donated blood samples were males of similar age (25 years old ± 1.0).

Howanitz et al study (8) lacks any correlation between the results obtained and the age of the volunteers who were used in their study. As illustrated in table (1) there was a significant difference ($P < 0.01$) between the ammonia concentration in heparinised plasma (mean value 36.6 ± 1.9 μmole/L) and those from EDTA - specimen (mean value 42.6 ± 2.3 μmole/L). While there was also a significant difference ($p < 0.01$) between ammonia values from heparinised specimen (mean value 36.6 ± 1.9 μmole/L) and that in serum specimen (52.6 ± 1.6 μmole/L). The data presented in this study was in agreement with the result obtained by other studies (8,10) that there is significantly less ammonia in heparinised and EDTA plasma than in corresponding serum. Since plasma specimen anticoagulated heparin or EDTA are recommended for ammonia determination (8), so far no study has been reported any comparison between ammonia value in EDTA and that in heparinised plasma. the reason for the remarkable decrease in ammonia concentration in heparinised plasma is due to the inhibitory effect of heparin on adenyl acid deaminase as reported by Kurahasi et al (11) which suggests that EDTA plasma is preferred over the other anticoagulant for ammonia determination. The EDTA collection tube has a slightly higher ammonia content (mean difference 3.0 μmole/L; $p > 1.0$) when half-filled than it did when completely filled (Table 2). This such observation suggests that the half filled tubes contain some air contaminated with ammonia which is subsequently dissolved in specimen. Contamination of this sort would be clinically insignificant. So in this regard proper precautions must be taken to avoid as much as we can the direct and long exposure of specimen to the atmosphere as reported by Gerron et al (4). Despite that the differences between the ammonia values in half-filled tubes and those in completely filled are small (Table 2), we do suggest to fill the collection tube which contains blood with

distilled water or deionized water to minimise air contamination, since ammonia concentration was too small (1.5 $\pm$ 0.06 µmole/L; 1.3 $\pm$ 0.06 µmole/L) in distilled and denionized water respectively (Table 3).

There were controversial reports about the effect of temperature on ammonia concentration in plasma. Some studies (12,13) reported that ammonia concentration is rapidly increased in plasma stored at 0 C^0 or frozen at -26 ^{0}C, but Howanitz et al. (8) found a slight increase (5.6%) in ammonia concentration when plasma specimen were stored for 1 hr at 4 ^{0}C , which suggests that the effect of temperature on the results obtained was negligible.

As illustrated in table (4) when EDTA specimen were inadequately centrifuged, the plasma ammonia levels were higher (mean difference 12.9 µmole/L; 31.0%; $P < 0.01$) than those in the adequately centrifuged EDTA tube. Cowley et al. (14) has found this to be ascribable to a high concentration of platelets in the unadequetely centrifuged specimen that would interfere with the enzymatic method used to determine the ammonia concentration and artefactually increase the ammonia concentration. Since erythrocytes contain about three fold as much ammonia as plasma (8), we found that the centrifugation at a higher speed ($> 1500 \times$ g) would cause some hemolysis to blood specimen and artefactually elevates the ammonia concentration (data is not reported). Most studies (3, 7, 8) used centrifugation forces at ($1500 \times$ g) to separate the plasma, but non of these has reported any comparison regarding the promptness of centrifugation speed.

In conclusion; the present study was carried out to investigate the effect of some factors on ammonia determination. From this study the following guide lines are given to ensure reliable plasma

ammonia results:

1. For the collection of the blood specimen, the use of EDTA tube is preferable, since heparinized and plain tubes will lower or elevate the ammonia concentration respectively.

2. Complete filling of the collection tube is preferable, but a partially filled specimen is also acceptable.

3. Smoking should be prohibited during blood collection because as reported by Gerron et al (4), that ammonia is so ubiquitous in tobacco smoke.

4. The blood specimen must be thoroughly mixed with anticoagulant. A clotted specimen must be rejected.

5. Adequate centrifugation of the blood specimen is essential (1500x g for 15 minutes).

6. If specimen can not be analysed promptly due to instrument malfunction or for any other reason, the specimen should be frozen at -20C^0 as reported by Howantiz et al. (8) that storage of specimen as plasma or as whole blood at 4 C^0 increases ammonia concentration greater than specimen stored at -20 ^{0}C or -70 ^{0}C.

7. Crossly hemolysed specimen should be rejected.

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Table 1: Ammonia concentration in serum and plasma specimen for ten volunteers (n = 10). The plasma specimens were obtained from the blood preserved in potassium citrate-EDTA and in lithium-heparinised tubes. The values are the mean of three measurements. Reference rage: 19-68 μmole/L (7).

Specimen No.	Ammonia concentration (μmole/L) ± SD					
	Serum	S.D	Potassium-EDTA	SD	lithium-heparin	SD
1	55.0	1.8	44.1	3.2	39.0	2.0
2	50.5	2.0	35.0	2.3	30.0	1.8
3	55.0	1.5	50.3	3.1	44.3	2.5
4	45.0	1.6	28.7	1.5	25.0	1.2
5	52.0	1.2	45.8	1.7	40.2	1.8
6	52.0	1.5	47.1	1.6	41.1	1.8
7	48.0	1.1	30.0	2.3	23.0	1.5
8	56.5	1.5	45.0	3.1	40.5	1.8
9	54.0	1.8	51.5	2.5	43.6	2.1
10	58.5	1.8	48.0	1.3	40.3	2.5
mean		(a)		(b)		(c)
	52.6	1.6	42.6	2.3	36.6	1.9

Statistical significant: a,b ($p < 0.01$)
b,c ($p < 0.01$)
a,c ($p < 0.01$)

Table 2: Effect of filling of the specimen potassium citrate EDTA-collection tubes. Ten paired specimens were used for the same group of volunteers. Each value is the mean of three measurements.

Specimen No.	Ammonia concentration (μmole/L) ± SD			
	Full tube	SD	Half tube	SD
1	43.2	2.1	47.0	1.2
2	32.1	1.5	36.5	2.1
3	45.0	3.2	40.3	2.2
4	31.0	2.6	29.6	1.1
5	48.2	1.1	55.2	1.8
6	44.0	2.5	48.3	1.6
7	31.0	3.1	33.0	2.1
8	47.2	1.2	52.0	1.9
9	48.0	3.0	52.6	2.2
10	50.1	1.8	55.5	2.8
mean		(a)		(b)
	42.0	2.2	45.3	1.9

Statistical significant a,b ($p > 5$)

Table 3: The concentration of ammoina in distilled and deionized water samples. The determination of ammoina was carried out in Du Pont ACA analyser. The SD was taked from three measurements.

No. of Sample	Ammonia concentration (μmole/L) ± SD			
	Distilled water	SD	Deionized water	SD
1	1.5	0.06	1.5	0.03
2	1.4	0.09	1.2	0.1
3	1.6	0.05	1.3	0.04
mean		(a)		(b)
	1.5	0.06	1.3	0.06

Statistical significant: a,b ($p > 2$).

Table 4: Effect of centrifugation on ammonia concentration. Five paired specimens from the same group of volunteers ($n = 5$) were used. Inadequately centrifuged specimens in potassuim citrate EDTA tubes were obtained at 500xg for 5 minutes, where as adequately centrifuged specimens were obtained at 1500xg for 15 minutes.

Specimen NO.	Ammonia concentration (μmole/L) ± SD			
	500 × g;5min.	SD	1500 × g; 15 min	SD
1	44.2	1.8	36.1	1.5
2	52.0	2.1	37.0	1.3
3	31.5	1.1	21.0	1.1
4	45.0	1.0	23.0	2.1
5	42.0	2.0	33.0	1.6
mean		(a)		(b)
	42.9	1.6	30.0	1.5

Statistical significant a,b ($p < 0.01$).

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