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Research Article

Effect of preanalytical techniques and variables on plasma ammonia determination

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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, promptness of centrifugation ,temperature and complete filling of blood collection tube. The use of non-hemolysed , non-clotted specimen and prompt centrifugation (1500 X g ; 15 minutes) apparently were important in avoiding such increase in ammonia concentration. Sodium citrated-plasma is preferred over the other anti-coagulated specimens for ammonia determination . Complete filling of the specimen tube has no significant effect on ammonia concentration. We concluded that the procedure for collection and handling of specimen for ammonia determination should be standardized and strictly observed.

Keywords: Ammonia, anti-coagulants, sodium citrated blood, Lithium- heparinized blood, centrifugation force.

Introduction

Blood collection and processing are two major steps in preanalytical laboratory testing. Proper blood collection and timely processing by well-trained staff using appropriate devices are needed to ensure test reliability. Accurate measurement of plasma ammonia is difficult because concentration in blood is low compared with values from potential contaminants from the laboratory environment or from endogenous source released during specimen handling and storage (Barsotti., 2001 and Haberle.,2011). Ammonia test is an important indicator for the diagnosis and follow of several hepatic and renal disorders (Garcia, 2011; Weiner et al,.2014). However since ammonia concentration easily changed with time during storage of sample, fresh samples should be analyzed immediately after collection (Howanitz et al.,1984). The effect of various conditions on ammonia determination was depated by Howanitz et al., 1984 and Erika et al,. 2012. Blood ammonia concentration decreased with age (1 year to 14 years) and the concentration of ammonia was found higher in men than in women (Meites.,1988 ; Diaz et al.,1995). There was a significant difference between concentration of ammonia in heparinized plasma and serum specimen (Lindner and Sandra., 1993). To fill the specimen tube completely requires a minimum of 6 to 8 ml of blood and this is often difficult in the case of critically ill babies (Leslie, 2005). Technical errors such as unproper mixing of blood with the anticoagulant leads to a slightly clotted sample which affect the level of ammonia in the sample (Cowley et al., 1985). In a busy emergency laboratory, the blood sample may not be adequately centrifuged which may affect ammonia concentration (Marjani, 2006).

The objective of this study was to investigate the effect of, types of anti-coagulated blood sample, prompt centrifugation and the filling of specimen, on the concentration of ammonia.

Materials and Methods

Samples

Venous blood samples from ten (n = 10) healthy volunteers (25 ± 1.0 years old) were collected into three types of collection tubes (sodium –citrated tube, lithium heparin tube and plain tube). These tubes were purchased from Greiner, Labtecnik Com.

Three groups of blood samples were collected from each volunteer and were immediately placed in ice.

Procedure of variable factors investigation

a. The effect of filling the samples in the collection tube: from paired collection tubes (n =10) , one was half filled and the other was fully filled with same blood sample.

b. The effect of centrifugation on the concentration of ammonia: using the second group of paired samples, one of each pair was centrifuged for five minutes at low speed (500 X g) and the other was centrifuged for 15 minutes at high speed (1500 X g) . Five paired samples were used in this study.

c. The effect of anticoagulated blood and coagulated blood samples: from each volunteer (n =10), three blood samples were withdrawn, one sample was properly mixed with sodium citrate anticoagulant , the other was mixed with lithium heparin anticoagulant while the third blood sample was placed in plain tube resulting in clotted blood samples .

Ammonia concentrations were measured in these set of samples with discrete analyzer ACA, with ammonia reagent packs both obtained from Dupont Instrument, Wilmington, DE.

Ammonia standard and control

40 and 80 µmole/L controls of ammonium chloride solutions were prepared from 400µmole/L of ammonium chloride solution (Howanitz et al., 1984). These controls were used to determine the instrument day –to- day precision.

Results and Discussion

The within day to day precision of the instrument for analytical variation was 2.5 % and 4.3 % for the 40 and 80µ/mole/L controls. Since there is difference in the concentration of ammonia between male and female (Diaz et al., 1995), all volunteers were males of similar age (25 ± 1.0 years old). As illustrated in table 1 there was significant difference ($p < 0.01$) between the ammonia concentration in heparinized plasma samples and those from sodium citrated plasma samples (($p < 0.01$). In general, ammonia concentrations in plasma samples obtained from anti-coagulated blood samples (Table 1) were significantly different from those in serum samples ($p < 0.01$). The data presented in this study was in agreement with the results reported by other studies (Barsotti, 2001 and Haberle, 2011) that there is significantly less ammonia in heparinized and sodium citrated plasma samples than in corresponding serum samples. The remarkable decrease in ammonia concentration in heparinized plasma is attributed to the inhibitory effect of heparin on adenyl acid deaminase enzyme (Kurahasi et al., 1972) which suggests that sodium citrate is preferred over the other anti-coagulant for ammonia determination. This result contradicts the report of Raffick et al., 2010 that heparinized and sodium citrated plasma samples are recommended for ammonia determination. As reported in table 2 the half filled sodium citrated blood collection tubes have a slightly higher ammonia concentration than one with completely filled (mean difference 3.3 µmole/L; $p > 1.0$). This such observation suggests that the half filled collection tubes contain some air contaminated with ammonia which is subsequently dissolved in the sample. Contamination of this sort would be clinically insignificant. Precautions must be taken to avoid the direct and long exposure of samples to the atmosphere (Gerron et al., 1976). Despite that the difference between the ammonia level in half filled collection blood tubes and these in completely filled are small (Table 2), it recommend to fill the blood collection tube with distilled or deionized water to minimize air contamination as ammonia concentrations (1.5 µmole/L ± 0.06; 1.3 µmole/L ± 0.026) in distilled and deionized are too small respectively (Table 3).

As illustrated in table 4 when sodium citrated plasma samples were inadequately centrifuged, the plasma ammonia levels were significantly higher than those in the adequately centrifuged sodium citrated plasma samples (42.9µmole/L; 30.0 µmole/L; $p < 0.01$ respectively) . Cowley et al., (1985) has found this to be ascribable to a high concentration of platelets in the inadequately centrifuged samples that would interfere with the enzymatic method used for determination of ammonia concentrations. Most studies (Howanitz et al., 1984; dariusz et al., 1992; Diaz et al., 1995; Haberle, 2011) used centrifugation force at 1500 X g to separate the plasma, however none of these has reported any comparison regarding the promptness of centrifugation speed.

Table 1. Ammonia concentrations in serum and plasma samples measured in ten volunteers (n = 10). The plasma samples were obtained from the blood preserved in sodium citrate and in lithium heparin. The values are the mean of three measurements. Reference range of ammonia: 19.0 – 68.0 µmole\ L.

Sample No.	Ammonia concentration (µmole\ L)					
	Serum	SD	Sodium citrate	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	40.0	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	52.6 (a)	1.6	42.6 (b)	2.3	36.6 (C)	1.9

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

Table 2. Effect of filling of the sodium citrate blood collection tubes on ammonia concentration. Ten paired samples from the same volunteers were used. The values are the mean of three measurements.

Sample No.	Ammonia concentration (µmole\ L)			
	Full filled collection tube	SD	Half-filled collection 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	42.0(a)	2.2	45.3(b)	1.9

Statistical significance: a,b ($p > 1.0$)

Table 3. The concentrations of ammonia in distilled and deionized water samples. The SD was taken from three measurements.

Sample No.	Ammonia concentration (µmole\ L)			
	Distilled water	SD	Deionized water	SD
1	1.5	0.06	1.5	0.03
2	1.4	0.09	1.2	0.01
3	1.6	0.05	1.3	0.04
Mean	1.5(a)	0.06	1.3 (b)	0.026

Statistical significance: a,b ($p > 2.0$)

Table 4. Effect of centrifugation on ammonia concentration. Five paired samples were used. Inadequately centrifuged sodium citrated blood sample at 500 X g for 5 minutes and adequately centrifuged one done at 1500 X g for 15 minutes. The SD was taken from three measurements.

Sample No.	Ammonia concentrations (µmole\ L)			
	500 X g	SD	1500 X g	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	42.9[(a)]	1.6	30.0 [(b)]	1.5

Statistical significance: a,b ($p < 0.01$)

Conclusion

The present study was carried out to investigate the effect of some factors on ammonia concentration. It is recommended to use the sodium citrated plasma as proper sample for the determination of ammonia levels. The blood sample must be thoroughly mixed with anti-coagulant and clotted sample should be rejected. Adequate centrifugation of blood samples is essential at 1500 X g. If samples cannot be analyzed for ammonia levels due to instrument malfunction or for any other reason, the samples should be frozen at -20C^{o} at which ammonia level is preserved (Howanitz et al., 1984)

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