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Precision Analysis, Analytical Standardization and Comparative Study of Hyperammonemia in Patients with Various Hepatic Anomalies

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Abstract *Background*: Hyperammonemia occurs in patients of hepatic anomalies, such as hepatic failure, intra or extra hepatic shunting or urea cycle enzyme deficiencies and chronic hepatitis. *Aim:* Present study describe correlation of hyperammonemia with various hepatic conditions, in addition to comparative precision analysis of blood ammonia from normal and pathological subjects by using manual semi-automated instrument and automated chemistry analyzer. *Materials and Methods*: It was a retrospective study covering the period of Dec 2012 to Dec 2016 and included 169 patients, with history of hepatic anomalies (cirrhosis, hepatic encephalopathy, alcoholic cirrhosis, hepatitis). Consolidated and completed data of ninety three males and seventy six female patients were assembled and classified on the basis of gender, age and blood ammonia ranges from normal to abnormal (9-26 μmol/L, 26-55 μmol/L and 55-100 μmol/L). Blood ammonia was analyzed on semi-automatic Randox Monza and fully automated Roche Cobas c501 by standardized method. Precision and standardization analysis was performed by using data of blood ammonia analyzed on Randox Monza for all three ranges on Cobas c501 and compared with each other by Regression correlation analysis R^2 on SPSS ver 15. Data were considered significant when $P< 0.05$. Results: Percent occurrence of normal blood ammonia level was 58.07% (n = 54) whereas for 26-55 μmol/L it was 26.89% (n = 25) and for further higher between 55-100 μmol/L was 15.06% (n = 14), which showed significant progression of hepatic anomalies. Similarly, in female group of both normal (60.53%, n = 46) and diseased subjects (n = 30), variations in blood ammonia levels corresponded to the severity of the disease or absence of it. Comparative precision analysis of blood ammonia performance on both Randox Monza (semi automatic) and automated Cobas c501 showed significant correlation, clearly manifested by $R^2 = 0.995$ (99.6%) in normal subjects, R^2 0.998 (99.8%) in pathological subjects of range 26-55 μmol/L and R^2 0.995 (99.5%) in pathological range 56-100 μmol/L. *Conclusion*: Our study clearly indicated the correlation between high blood ammonia levels at various pathological ranges, both in male and female patients. Moreover comparative precision analysis of two separate analyzers provided significant baseline data to suggest quality controlled compatible methodology and instrumentations.

Keywords Precision analysis, hyperammonemia, automated, semi-automated

1. Introduction

Hyperammonemia is a condition characterized by increase in the level of blood ammonia [1]. As a resultant of hyperammonemia, excess ammonia circulates in the body and body fluids and covert into urea in liver [2]. Ammonia, which is a water soluble highly volatile alkaline substance, is generated through oxidative deamination of amino acids, and conversion of other nitrogenous compounds into ammonia [3]. It is well documented that hyperammonemia takes place in patients suffering from hepatic anomalies, such as hepatic failure, intra or extra hepatic shunting or urea cycle enzyme deficiencies and chronic hepatitis [4-6]. Hyperammonemia stated to be the main cause of hepatic encephalopathy and thus in such cases, blood ammonia analysis became imminent within shortest span of time with established accuracy [1,2,7,8].

Present study describe the correlation between high blood ammonia levels at various pathological ranges, both in male and female patients as well as comparative precision analysis of blood ammonia from normal and pathological subjects by using manual semi-automated method on Monza analyzer (Randox, UK) and automated Roche Cobas c501 (Roche, Basil).

2. Materials and Methods:

2.1. Patient selection and research design: It is a retrospective study covering the period of Dec 2012 to Dec 2016. A total of 169 patients, with history of hepatic anomalies (cirrhosis, hepatic encephalopathy, alcoholic cirrhosis, hepatitis) were assessed for presented study and then data were retrieved from LIS and HIMS. Consolidated and completed data, lab investigations, CT, diagnoses of ninety three males and seventy six female patients were assembled, include and classified accordingly. Final grouping was based on gender, age and blood ammonia ranges from normal to abnormal (9-26 μmol/L, 26-55 μmol/L and 55-100 μmol/L; details in Table 1 and 2). Age and gender matched samples were also included from patients with no sign of any hepatic anomalies or hepatic surgical interventions.

Table 1: Blood ammonia levels (μmol/L) in male subjects with normal controls (n = 54) and patients (n = 39) with underlying pathological conditions (cirrhosis, hepatic encephalopathy, chronic hepatitis)

	Blood ammonia range groups		
Patients/subjects age groups	**9-26 μmol/L (normal)**	**26-55 μmol/L (Pathological)**	**55-100 μmol/L (Pathological)**
20-35 yrs (n = 32)	13.15 ± 2.50 (n = 17)	36.15 ± 10.15 (n = 10)	86.20 ± 13.45 (n = 5)
36-50 yrs (n = 25)	14.40 ± 3.20 (n = 15)	44.45 ± 9.80 (n = 6)	79.85 ± 12.55 (n = 4)
51-70 yrs (n = 21)	12.75 ± 3.65 (n = 13)	41.30 ± 9.65 (n = 5)	88.40 ± 15.30 (n = 3)
> 71 yrs (n = 15)	13.55 ± 3.10 (n = 9)	39.85 ± 10.25 (n = 4)	81.55 ± 14.70 (n = 2)
Total N = 93	N = 54 (58.07%)	N = 25 (26.89%)	N = 14 (15.06%)

Results are expressed as mean ± SD

Table 2: Blood ammonia levels (μmol/L) in female subjects with normal controls (n = 46) and patients (n = 30) with underlying pathological conditions (cirrhosis, hepatic encephalopathy, chronic hepatitis)

	Blood ammonia range groups		
Patients/subjects age groups	**9-26 μmol/L (normal)**	**26-55 μmol/L (Pathological)**	**55-100 μmol/L (Pathological)**
20-35 yrs (n = 25)	12.20 ± 3.40 (n = 15)	32.20 ± 11.20 (n = 7)	75.15 ± 14.10 (n = 3)
36-50 yrs (n = 24)	13.10 ± 4.10 (n = 15)	41.30 ± 10.25 (n = 6)	80.65 ± 13.65 (n = 3)
51-70 yrs (n = 15)	14.05 ± 4.05 (n = 9)	38.85 ± 10.45 (n = 4)	81.70 ± 14.55 (n = 2)
> 71 yrs (n = 12)	12.65 ± 4.25 (n = 7)	36.70 ± 11.50 (n = 3)	78.35 ± 13.30 (n = 2)
Total N = 76	N = 46 (60.53%)	N = 20 (26.32%)	N = 10 (13.16%)

Results are expressed as mean ± SD

2.2. Blood ammonia analysis: Blood ammonia was analyzed on semi-automatic Randox Monza and fully automated Roche Cobas c501 by the method described earlier [7]. The principle is based on combination of blood ammonia in the presence of glutamate dehydrogenase (GLDH) to yield glutamate and NADP+. The corresponding decrease in

absorbance at 340nm is proportional to the plasma ammonia concentration. Other parametric data such a urea, creatinine, amylase, liver function tests (CBC), hepatitis profile were also evaluated for confirmation of hyperammonemia, but not included in final data presentation because of non-relevance to objective of the study.

2.3. Precision and standardization analysis: Data of blood ammonia analyzed on Randox Monza for all three ranges were analyzed on Cobas c501, with 10-20 run of each sample. Data generated from both instruments were compared with each other by Regression correlation analysis R2 on SPSS ver 15. Data were considered significant when P< 0.05.

3. Results

Results are summarized in Table 1 and 2 and Fig 1-3. In male gender, patients and control subjects are divided into four age groups, 20-35, 36-50, 51-70 and above 70 years. Percent occurrence of normal blood ammonia level was 58.07% (n = 54) whereas for 26-55 μmol/L it was 26.89% (n = 25) and for further higher between 55-100 μmol/L was 15.06% (n = 14), which showed significant progression of hepatic anomalies corresponding to equally matched blood ammonia levels (Table 1). Similarly, in female group (Table 2) of both normal (60.53%, n = 46) and diseased subjects (n = 30), variations in blood ammonia levels corresponded to the severity of the disease or absence of it. In the pathological range of 26-55 μmol/L 26.32% (n = 20) showed an average of 32.20 ± 11.20 μmol/L to 41.30 ± 10.25 μmol/L, whereas in the range of 55-100 μmol/L, 13.16% (n = 10) showed 75.15 ± 14.10 μmol/L to 81.70 ± 14.55 μmol/L blood ammonia levels (Table 2).

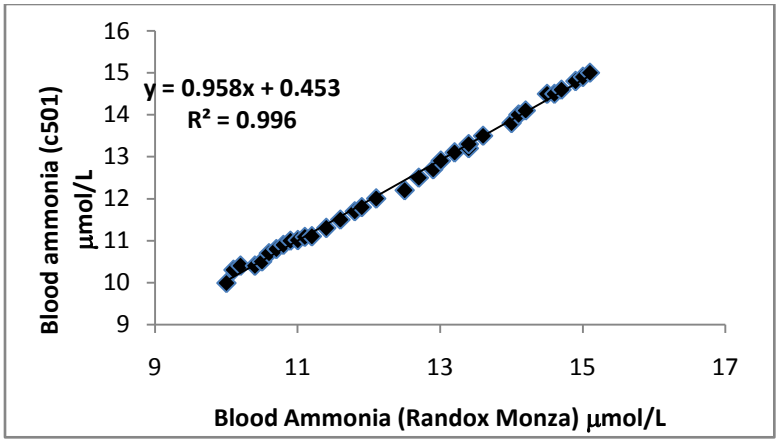

Figure 1: comparative precision analysis of normal Ammonia levels on Randox Monza vs c501 (n = 35)

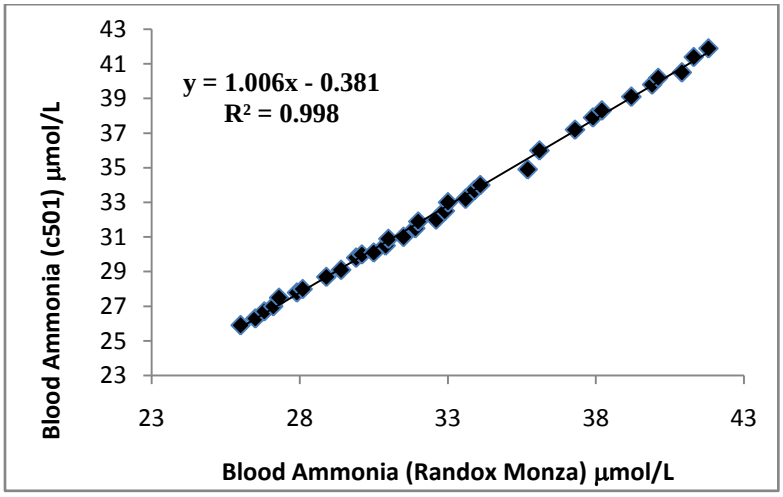

Figure 2: Comparative precision analysis of Pathological Ammonia levels on Randox Monza vs c501 (n = 35)

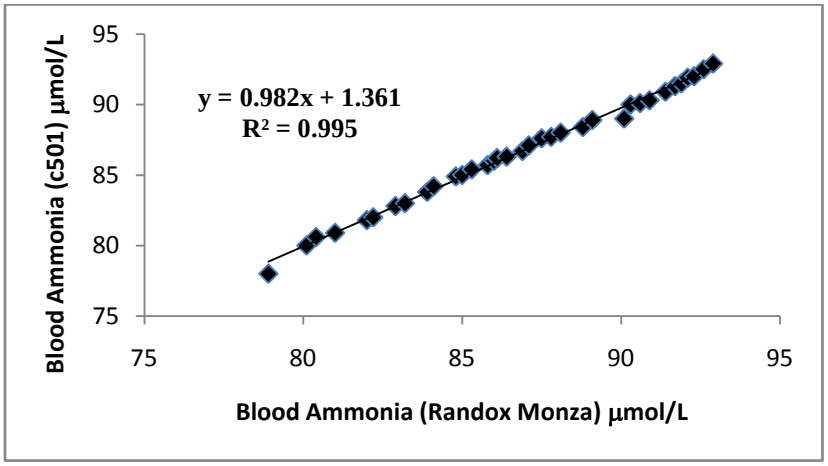

Figure 3: Comparative precision analysis of Pathological Ammonia levels Randox Monza vs c501 (n = 35)

Comparative precision analysis of blood ammonia performance on both Randox Monza (semi automatic) and automated Cobas c501 showed significant correlation, clearly manifested by R^2 = 0.995 (99.6%, Fig 1) in normal subjects, R^2 0.998 (99.8%) in pathological subjects of range 26-55 µmol/L (Fig 2) and R^2 0.995 (99.5%) in pathological range 56-100 µmol/L.

4. Discussion

Aim of our study was to evaluate and compare existing methods of blood ammonia determination, on both semi-automated Randox Monza (Randox) and fully automated Cobas c501 analyzers. In addition, comparative precision analysis was also performed to asses the blood ammonia determination method's compatibility and reproducibility of semi-automated and fully automated analyzers. Our study showed considerable regression correlation of method and precision on both analyzers with significant data relation with disease condition and hyperammonemia levels. Commercial clinical labs generally use glutamate dehydrogenase-catalyzed reactions where corresponding decrease in absorbance at 340nm is proportional to blood ammonia concentration [3], and in our study, both instruments exhibited proportionate analyzing activity and precision.

Hyperammonemia is a condition which has been attributed to the development of various hepato-pathological conditions, such as hepatic encephalopathy, cirrhosis, chronic liver disease, alcoholic hepatitis and viral hepatitis [3,9-11]. Hyperammonemia itself can induce complex neurological anomalies from changes in behavior, partial consciousness and abnormal neuromuscular function [9,11]. For any severe pathological changes taking place in hepatic system, hyperammonemia is the foremost indicator of cirrhosis, inflammation, chronic viral infections or encephalopathy [11]. For any correlation study, corresponding clinical condition with equally matched blood levels of biochemical components is a necessity and endorses clinical and management decisions. Moreover, analytical method selection, instrumentation as well as its precision is equally significant to ensure quality assured reproducible results whether done on semi-automated or automated instruments. Reproducibility ensures clinicians faith on lab results, in addition to patients satisfaction and better sustainable prognostic outcome.

5. Conclusion

Our study clearly indicated the correlation between high blood ammonia levels at various pathological ranges, both in male and female patients. Similarly, hyperammonemia also noted to be corresponding to prevailing hepatic patho-physiology in all patients. Moreover comparative precision analysis of two separate analyzers provided significant baseline data to suggest quality controlled compatible methodology and instrumentation. Thus performing analysis

on semi-automated analyzer or shifting from semi-automated method to a fully automated instrument showed no deviation and former is suggestively more efficient and financially feasible.

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