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Original Article

Biochemistry Section

Delay in Specimen Processing-Major Source of Preanalytical Variation in Serum Electrolytes

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ABSTRACT

Objective: To evaluate the stability of electrolytes in serum samples due to delay in analysis in a tertiary care government hospital in India, and the maximum time delay acceptable between sample centrifugation and analysis.

Materials and Methods: We estimated serum electrolytes of 400 samples with different time intervals between centrifugation and sample analysis on automated analyser.

Results: Values were compared using repeated measure ANNOVA and acceptable limit change using in house QC values of 6 months. During the time interval between centrifugation and sample analysis, the samples were kept uncovered in sample cups in the laboratory. Potassium values show significant changes within 1 h (T1, $p<0.01$) but sodium (T2, $p<0.01$) and chloride (T2, $p<0.001$) values are acceptable up to a time delay of 3 h between sample centrifugation and analysis.

Conclusion: Samples for electrolytes should be analysed within 1-2 h of centrifugation and if there is any delay in analysis, the samples should be stored under proper conditions.

Keywords: Acceptable limit change, Serum electrolyte, Sample stability

INTRODUCTION

Laboratories play a pivotal role in enabling clinicians to make a correct decision. According to a survey, around 60-70% of the decisions about admissions, discharges and medications are based upon laboratory results [1]. Laboratory testing is a complex process, involving a series of inter related steps each step being prone to error. According to the draft of the ISO technical report 22367, laboratory error is defined as "a defect occurring at any part of the laboratory cycle, from ordering tests to reporting results and appropriately interpreting and reacting to these" [2]. Laboratory error leads not only to unnecessary delays and additional costs by necessitating obligatory repeat samples but also imparts unnecessary pain to the patient. Laboratory testing is divided into three phases-preanalytical, analytical and post analytical. Most errors occur in the preanalytical phase (46-68.2%) and the post analytical phase (18.5-47%) but still a large fraction (4-32%) can be attributed to the intra-analytical phase of the testing process [3]. Laboratory errors in the analytical phase have significantly decreased in recent times due to automation and technological advancements.

The transition phase between the preanalytical and the intra analytical phase is also considered to be an important error prone area. This phase is usually under the direct control of the laboratory but precedes the time in which analyte is analysed. Analyte stability time described by WHO [4] and CLSI [5] is often difficult to apply in the clinical settings as the time taken to transport samples from collection centre to laboratory and the time interval between centrifugation and processing is usually a laboratory dependent variable.

Common investigations ordered by the clinicians are primarily blood sugar, liver function test, and kidney function test and serum electrolytes. Electrolytes which include sodium, potassium and chloride dictate not only the management protocol but also the outcome. Sodium, the major extracellular cation is regulated by the kidneys. Hypo/hyper natrimia is associated with excessive urine loss, diarrhea, Addisons disease, renal tubular disease, severe dehydration, brain injury, diabetic coma and excessive intake of sodium salt [6]. Potassium on the other hand, is the major intracellular cation. Measurement of serum potassium is used for evaluation of electrolyte imbalance, cardiac arrhythmia, muscular weakness, hepatic encephalopathy and for monitoring of ketoacidosis in diabetes mellitus and intravenous fluid replacement therapy [7]. The physiological significance of chloride, anion of extracellular water space, is in monitoring proper body water distribution, osmotic pressure and normal anion cation balance in the extracellular fluid compartment [8]. Spurious elevations of these electrolytes which do not correspond to the state of the patient are common findings in the laboratory. But when these specimens are recollected and re-analysed, these values drop substantially.

In our hospital, sample collection is done in the Common Collection Centre for the out patients by trained laboratory phlebotomists. The time for sample collection for the out patients department is from 9.00am to 11.30am. These samples are then transported from the common collection centre to laboratory by trained laboratory staff. The samples are then centrifuged in the laboratory, and serum is transferred into the sample cups for analysis on fully automated clinical chemistry analyser. At times there is a delay in sample analysis after centrifugation due to a large sample load, breakdown of the analyser and the non availability of a backup system.

We therefore sought to evaluate if this time lag between centrifugation and sample analysis has any effect on the stability of electrolytes in serum and in turn on the final values reported by the laboratory.

MATERIALS AND METHODS

This study was conducted in the Department of Biochemistry, PGIMER and Dr. RML Hospital, New Delhi, India. All the samples were collected from OPD patients. The samples were collected by trained phlebotomist of the department. All phases of the sample collection were accurately standardized including identical resting times for the subjects (>5mins), tourniquet placement (<30sec) and the use of vacuum tubes from the same lot [9]. The tourniquet was released after the blood began to flow in the tube. Venous blood was collected by needle (22GA x 1", CAT NO-301747) and adapter and separated into 3.5mL serum separator tubes with an inert polymer gel barrier and a clot activator (Becton Dikinson, Franklin Lakes, NJ, USA, cat no-367954). After allowing the sample to clot for 30 min, the samples were centrifuged in the laboratory and transferred to the

	mean	SD	minimum	median	Maximum
Sodium (T0)	142.6mmol/l	4.7	124mmol/l	144mmol/l	147mmol/l
Sodium (T3)	147.46mmol/l	5.6	128mmol/l	147mmol/l	158mmol/l
Potassium(T0)	4.56mmol/l	0.5	3.86mmol/l	4.66mmol/l	5.56mmol/l
Potassium(T3)	4.76mmol/l	0.5	4.6mmol/l	4.76mmol/l	5.96mmol/l
Chloride(T0)	101 mol/L	4.5	87 mol/L	103 mol/L	106mol/L
Chloride(T3)	110 mol/L	6.2	92 mol/L	112 mol/L	118mol/L

[Table/Fig-1]: Electrolyte concentration at 0hrs and 3hr

Sodium	Mean Difference	Q	p	Confidence interval
T0-T1	-1.64	3.48	>0.05	-3.39to0.10
T0-T2	-4.77	10.11	<0.001	-6.52to-3.02
T0-T3	-8.22	17.41	<0.001	-9.97to-6.47
Potassium				
T0-T1	-0.11	5.55	<0.01	-0.18to-0.03
T0-T2	-0.22	11.2	<0.001	-0.30to-0.15
T0-T3	-0.44	22.05	<0.001	-0.30to-0.15
Chloride				
T0-T1	-1.13	2.11	>0.05	-3.27to1.00
T0-T2	-5.26	9.83	<0.001	-7.40 to-3.13
T0-T3	-8.93	16.68	<0.001	-11.07 to -6.79

[Table/Fig-2]: Comparison of electrolyte at different time interval

Analyte	Acceptable Change Limit	T1	T2	T3	T4
Sodium	4.15	1.6	2.59	4.34	16.9
Potassium	6.09	3.1	4.38	7.7	19.3
Chloride	7.47	1.2	5.1	8.69	18.9

[Table/Fig-3]: Comparison between acceptable change limit with values at different time period

sample cups for analysis. The samples were analysed within 30mins (T0), 1 h(T1), 3 h(T2), 5 h(T3) and 24 h(T4) after centrifugation of samples. During this period the samples were kept in the laboratory in sample cups, uncovered, unrefrigerated at a room temperature of 28-32°C. After 5 h the samples were refrigerated at 4°C to be analysed next day.

The samples were analysed on Vitros 5.1, fully automated dry clinical chemistry analyser (Ortho Clinical Diagnostics, Inc, Rochester, NY, USA). The control values were within range during the analysis period and we ruled out any analytical variation as the precision for last six months was in the acceptable range. Exclusion criteria chosen for the samples was hemolytic index (HI) >15. This analyser automatically measures HI in all blood samples using a spectrophotometric technique [10,11]. Light from the lamp travels by fibre optics through the sample. The pickup fibres channels the light to the spectrophotometer. The computer calculates the amount of light transmitted versus the amount the light absorbed to measure the interference and calculates the concentration. 1g/dl of hemoglobin corresponds to HI of 99. The samples were considered to be hemolysed at a HI ≥15 (equivalent to 0.15g/L of free hemoglobin)

In vitros 5.1 automated dry chemistry analyser, electrolyte analysis is performed using slides, with vitros chemistry products calibrator kit 2. The slides for electrolytes are multilayered analytical element coated on a polyester support that uses direct potentiometry for measurement of different ions [12]. The slide consists of two ion selective electrodes, each containing a reference layer, silver and a silver chloride layer coated on a polyester support. For potassium the slide contains valinomycin, for sodium methyl monensin and for chloride a protective layer in addition to the above. A drop of patient sample and a drop of vitros reference fluid on separate halves of the slide results in migration of both fluid towards the centre of the paper bridge. The potential difference between the two electrodes is proportional to the electrolyte concentration in the sample.

STATISTICS

SPSS 19.0 for windows (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. The change in concentration after a delay in processing was measured by repeated measure ANOVA. The significance level was set up at $p<0.05$. The mean percentage deviation was compared to the acceptable change limit (ACL) according to ISO 5725-6 [13]. The ACL for interpreting a measured difference was based on the analytical imprecision (CV) using the formula ACL=2.77 x CV [14]. The CV was obtained from in house routine mean data of QC value over 6 months duration.

RESULTS

Of the 400 samples analysed 152(38%) are of women and 248(62%) of men. The women on an average are 37 years old and men 45years old. In women, the mean sodium concentration is 144.2mmol/l, potassium 4.7mmol/l and chloride 101mol/L at 30mins (T0). In men, the mean sodium is 141.5mmol/l, potassium 4.4mmol/l and chloride 105mol/L at 30mins(T0).

[Table/Fig-1] shows the mean electrolyte concentration in the samples at 0 h (T0) and 3 h(T3) after centrifugation. All the electrolytes show an increasing trend with delay in processing after centrifugation. All the samples were kept uncovered in the laboratory during this period. Similar results are obtained in the samples processed beyond 3 h.

[Table/Fig-2] shows the mean difference, p-values and confidence intervals when results obtained for samples immediately after centrifugation (T0) are compared with results from different time intervals. Sodium clearly shows a significant difference at 3 h(T2) and 5 h (T3) with a p-value of <0.001 for both. Potassium however shows a significant difference even at 1 h(T1)($p<0.01$). For chloride, significant difference is observed only at 3h (T2) ($p<0.001$) and 5h (T3) ($p<0.001$). Values at 24 h are not shown as they show the same trend.

[Table/Fig-3] shows the comparison between acceptable change limit with sodium, potassium and chloride. The acceptable change limit is calculated by 2.77 x CV. The CV is obtained by in house QC values of 6 mnth with 1.5 for sodium, 2.2 for potassium and 2.7 for chloride. This table shows that after 5 h (T3) values for sodium, potassium and chloride exceeds the acceptable change limit.

DISCUSSION

Various studies have been conducted in the past to demonstrate the stability of many analytes. Donnelly et al., who investigated the stability of 25 analytes showed that sodium, potassium and chloride remain stable for 24 h at room temperature, 4°C and -20°C [15]. Bobby et al., who investigated the stability of 24 analytes after prolonged contact of plasma and serum with blood cells and after immediate separation of plasma and serum at room temperature (25°C) and analysed in 0,2,4,8,16,24,32,40,48 and 56 h after collection found the sodium, potassium and chloride remain stable up to 56 h [16]. Similarly Heins at al., who performed stability studies on 22 serum analytes found out that electrolytes remain stable after 24 h [17].

However study by Tanner et al., on 30 adult healthy volunteers on 35 analytes showed that stability of potassium is altered within 24 h but sodium remains stable up to 24 h [18]. In all the studies on analyte stability strict temperature maintenance is followed.

Our study which is performed in a tropical country (India), temperatures sometimes surge upto 46°C in summers. Despite the very high temperatures, we try to maintain the ambient temperature in the laboratory at 25-30°C with the help of air conditioners and

fans. Our study shows that stability of sodium, potassium as well as chloride is altered after few hours of centrifugation if there is a delay in analysis. Sodium and chloride results are affected at 3 h (T2) but potassium results are affected even earlier at 1 h (T1). Probably this gross difference between other stability studies and our study is due to improper temperature maintenance in laboratory leading to significant evaporation from sample cups. Climatic conditions as well as uncovered sample cups left under the fan for a few hours are responsible for this evaporation and falsely high serum electrolyte values.

CONCLUSION

This study shows the existence of various preanalytical variables prevailing in government hospitals in India where a large patient load alongside scarcity of resources (manpower, equipment, analysers, proper storage facilities), at times lead to inaccurate patient reports. The existence of various shortcomings and the paucity of adequate and proper facilities in most of the Government Hospitals in our country are brought to the surface by this study. To conclude we suggest that the samples for measurement of serum electrolytes should be analysed as soon as they are received in the laboratory preferably within 1-2h. In the event of any delay sample cups should be properly covered and stored under proper condition. We assume that the outcomes are applicable to large number of government hospitals in India. Follow up analysis on a large population would be of further help.

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