



Effect of serum clot contact time as a major source of preanalytical variation in serum electrolytes

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Abstract:

Introduction: Electrolyte measurement especially for sodium and potassium are frequently required for patients admitted in the hospital. Serum electrolytes are most affected parameters by preanalytical variables like serum clot contact time.

Aim: To evaluate if the time lag between centrifugation and sample analysis has any effect on the stability of electrolytes in serum. **Material & Methods:** The study was conducted in the department of biochemistry, Navodaya medical college Hospital and research centre, Raichur, Karnataka. Fifty blood samples were drawn in replicates and brought to the laboratory. One batch of the samples was processed immediately and serum was separated for electrolyte analysis i.e. Na, K and Cl. The second batch of replicate samples were processed after three hours and assay performed. **Results:** The mean Sodium, Potassium and Chloride concentration were 137.92±4.97mmol/L, 4.44±0.51mmol/L and 105.64±7.26 mmol/L at 30 min (T0). The mean Sodium, Potassium and Chloride concentration after 3 hours of serum clot contact time were 138.62±5.46mmol/L, 4.52±0.53mmol/L and 105.92±7.45 mmol/L (T3). All the electrolytes show an increasing trend with delay in processing. Both sodium and potassium clearly shows a significant difference at T0 (30 min) and T3 (3 hours) with a p value of <0.001 for both. For chloride, the p-value was insignificant (0.056). **Conclusion:** The stability of both the electrolytes (sodium and potassium) is found to be sensitive to prolonged serum clot contact time. Thus it is essential that the blood samples obtained from the patients be processed timely and the analysis done immediately.

Key words: Serum electrolytes, Serum clot contact time, preanalytical Variables, Sodium, Potassium

Introduction:

Electrolyte abnormalities are one of the common reversible causes of morbidity and mortality in Intensive Care Unit (ICU) patients [1]. Accurate estimation of serum electrolytes has gained importance in diagnosis of etiology of various diseases as it is used to calculate Anion Gap. Electrolyte abnormalities can precipitate life threatening events by derangements in its metabolic process or as a consequence of an underlying disease.

Electrolyte analysis includes sodium, potassium and chloride. Sodium is the major cation of extracellular fluid. Its primary function in the body is to chemically maintain osmotic pressure and acid base balance and to transmit nerve impulse. Hyponatremia may be associated with sodium losses due to vomiting or diarrhea, diuretics abuse, salt losing nephropathy & osmotic diuresis, metabolic acidosis, adrenocortical insufficiency, dilution type hyponatremia may be due to edema, cardiac failure,

hepatic failure and hypothyroidism. Hypernatremia is associated with conditions with water loss in excess of salt through profuse sweating, severe vomiting or diarrhea, diabetes insipidus or diabetic ketoacidosis, hyperaldosteronism, Cushing's syndrome, inadequate water intake because of coma or hypothalamic disease, dehydration or excessive saline therapy.

Potassium is the major intracellular cation. Potassium plays a role in nerve conduction, muscle function and helps maintain acid-base balance and osmotic pressure. Measurement of serum potassium is used for evaluation of electrolyte imbalance, cardiac arrhythmia, muscular weakness, hepatic encephalopathy, monitoring of diabetic ketoacidosis and intravenous fluid replacement therapy.

Chloride is an anion that exists predominantly in extracellular spaces. It maintains cellular integrity through its influence on osmotic pressure. It is also significant in monitoring acid base and water balance. It is used in monitoring proper

body water distribution, osmotic pressure and normal anion cation balance in the extracellular fluid component [2].

Laboratory testing is divided into three phases, pre-analytical, analytical and post-analytical. Laboratory errors in the analytical phase have significantly decreased due to automation. Most errors occur in pre-analytical phase i.e. 46-68.2 % [3]. Among the investigations performed in the laboratory, the parameters which are mostly affected by these pre-analytical variables are the serum electrolytes [4]. The time interval between blood collection and sample processing in analyzer is one of the most error prone areas and also the bottleneck of the turnaround time of the laboratory. The two important time delay processes that occur in this phase is serum clot contact time and centrifugation delay. Serum clot contact time is defined as “optimum time interval between sample collection and separation of serum from the clot”. This period should be long enough to allow the complete clot formation but should be shorter than the time in which a significant change in the test result is induced by serum-clot contact. A minimum time interval of 20-30 min between blood collection and serum separation is considered acceptable [5].

A prolonged contact time between serum and clot alters both biological activities of the cells and trans-membrane diffusion that can change the concentration of serum electrolytes.

Although the practice in clinical biochemistry is to assay the samples as they are brought into the laboratory and after separation of the serum from the samples, at times the samples reach the laboratory from the wards and collection centers unduly late. Also at times the samples are processed inadvertently very late and also the assays may be missed and have to be done many hours after they have been collected due to large sample load, breakdown of the electrolyte analyzer and the non availability of a backup system. Information on the measured concentration of serum electrolytes during storage and serum clot contact time is often incomplete and sometimes contradictory.

Therefore we conducted this study to evaluate if the time lag between centrifugation and sample analysis has any effect on the stability of electrolytes in serum. Our study was designed to provide information relevant to our current testing method on immediate separation versus delayed centrifugation before analysis.

Materials and Methods:

The study was conducted in the department of biochemistry, Navodaya medical college Hospital and research centre, Raichur, Karnataka. Study was approved by institutional ethical committee and informed consent was obtained from the patients. Venipuncture is carried out on patients by trained laboratory technician of the department. Resting time of 5 min and tourniquet placement time of 30 sec is employed for each collection. Fifty blood samples were drawn in replicates and brought to the laboratory. One batch of the samples was processed immediately and serum was separated for electrolyte analysis i.e. Na, K and Cl. The second batch of replicate samples were processed after three hours and assay performed.

All the samples are analyzed by Ion selective electrodes in the AVL 9180 electrolyte analyzer. There are four different electrodes in the electrolyte analyzer i.e. sodium, potassium, chloride and reference electrode. Each electrode has an ion-selective membrane that undergoes a specific reaction with the corresponding ions contained in the sample being analyzed. The membrane is an Ion exchanger, reacting to the electrical charge of the ion causing a change in the membrane potential or measuring voltage which is built up in the film between the sample and the membrane. A galvanic measuring chain within the electrode determines the difference between the two potential values on either side of the membrane. The galvanic chain is closed through the sample on one side by the reference electrode, reference electrolyte and the “open terminal”. The membrane, inner electrolyte and inner electrode close the other side. A difference in ion concentration between the inner electrolyte and the sample causes an electro chemical potential to form across the membrane of the active electrode. The potential is conducted by a highly conductive inner electrode to an amplifier. The reference electrode is connected to ground as well as to the amplifier. The ion concentration in the sample is then determined by using a calibration curve determined by measured points of standard solution with precisely known ion concentration.

Statistics

Data analysis was done by using SPSS Software 19.0 for Windows. Comparison was done by using paired t test. Data is presented in terms of mean and SD. P value $p < 0.001$ is considered highly significant.

Results:**Table No:-1 Comparison of electrolyte concentration at T (0) and T (3) hrs.**

Parameter	Number of samples	Mean	SD	t-value	df	p-value	Inference
Sodium(T0)	50	137.92	4.97	-4.314	49	.0001	Highly significant
Sodium(T3)	50	138.62	5.46			(<0.001)	
Potassium(T0)	50	4.444	0.51	-4.390	49	.0001	Highly significant
Potassium(T3)	50	4.518	0.53			(<0.001)	
Chloride(T0)	50	105.64	7.26	-1.958	49	.056	Not significant
Chloride(T3)	50	105.92	7.45			(>0.05)	

A total of 50 samples were analyzed, of which 38(76%) are of men and 12(24%) were of women. The mean Sodium, Potassium and Chloride concentration were 137.92 ± 4.97 mmol/L, 4.44 ± 0.51 mmol/L and 105.64 ± 7.26 mmol/L at 30 min (T0).

The mean Sodium, Potassium and Chloride concentration after 3 hours of serum clot contact time were 138.62 ± 5.46 mmol/L, 4.52 ± 0.53 mmol/L and 105.92 ± 7.45 mmol/L (T3).

All the electrolytes show an increasing trend with delay in processing after centrifugation. Both Sodium and Potassium clearly shows a significant difference at T0 (30 min) and T3 (3 hours) with a p value of <0.001 for both. For chloride, the p-value was insignificant (0.056).

Discussion:

Serum electrolytes are the most common analytes requested in the clinical biochemistry laboratory. It is a standard practice to process the serum immediately after the blood specimens reach the laboratory and proceed with the assay. Sometimes the samples arrival in the laboratory is delayed due to transport from the collection centre to the central lab. The analysis may also be delayed due to the increased work load or the casual attitude of the technicians. The adverse effects of prolonged serum-clot contact time were known long back and immediate separation of the serum from cells was advised. During a prolonged serum clot contact time,

both the biological activity of the cells and transmembrane diffusion can change the concentration of serum electrolytes. The current recommendation of an acceptable time interval between sample collection and serum separation is 2 hours [6].

Information on the stability of serum electrolytes during storage of serum is often incomplete and sometimes contradictory. Donnelly et al., Who investigated the stability of 25 analytes, showed that sodium, potassium and chloride remain stable for 24 hrs at room temperature, 4°C , and -20°C [7]. Heins et al., who performed stability studies on 22 serum analytes, found that electrolytes remain stable even after 24 hrs [8]. According to Rashmi rasi Datta et al., stability of electrolytes (sodium and potassium) is not altered when samples are stored at 23°C . The maximum clot contact time which has no effect on the stability of electrolytes is 3 hrs [9]. However, according to Baruah A et al., samples for electrolytes should be analyzed within 1-2 hrs of centrifugation and if there is any delay in analysis, the sample should be stored under proper condition [10].

In this study, we tried to find the effect of serum clot contact time on the measured concentration of serum electrolytes. The serum potassium values increases with serum clot contact time. The change becomes highly significant by 3 hrs at room temperature. Glycolysis was dominant

initially, so the potassium from the serum went inside the cells thus lowering the potassium values. But as time went on, the small amount of glucose present in the serum was depleted and passive diffusion of potassium from cells due to cell membrane disruption becomes dominant, producing an increase in potassium after longer serum clot contact time. This finding is in agreement with the report by Zhang et al and Datta RR et al [9, 11].

Most of the ATP our cells produce is used up to maintain the correct balance of electrolytes between the cell and its external medium [12]. The glucose in our sample is consumed by Glycolysis and the ATP generated is necessary to activate the pump to transport potassium inside the cells. The increase in serum sodium concentration is found to be significant after 3 hrs duration. This is due to increased activity of sodium potassium pump which is active while Glycolysis is dominant and due to passive diffusion from the cells after the cell membrane disruption. However the change in serum chloride value is not significant statistically.

Conclusion

The stability of both the electrolytes (sodium and potassium) is found to be sensitive to prolonged serum clot contact time. Thus it is essential that the blood samples obtained from the patients be processed timely and the analysis done immediately. Delay in assays following prolonged serum clot contact will give us erroneous results. These changes can be prevented by separating the serum within one hour after the blood has been collected. Because of practical and economic constraints only a relatively small number of patient specimens could be tested. We suggest that further studies with large sample size are needed in this field.

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Conflicts of interest:

The authors declare that there is no conflict of interests.

References:

1. Lee JW, Fluid and electrolyte disturbances in critically ill patients. *Electrolyte blood press.* 2010; 8: 72-81.
2. Burtis CA, Ashwood ER, Bruns DE Tietz textbook of clinical chemistry and molecular diagnostics, 4th Edition Philadelphia PA: WB Saunders, 2006:984-90.
3. Plebani M. Errors in Clinical Laboratory or errors in laboratory Medicine? *Clin. Chem Lab Med.* 2006; 44 (6):750-59.
4. Heins M, Heil W. Withold W. Storage of serum or whole blood sample? Effects of time and temperature on 22 serum analysts. *Eur J Clin Chem Biochem* 1995; 33:231-238.
5. Burtis CA, Ashwood ER, Bruns DE, Tietz textbook of clinical chemistry and molecular diagnostics. 4th edition Philadelphia PA: WB Saunders 2006:56.
6. Laessig RH, Indriksons AA, Hassemmer DJ, Paskey TA, Schwarts TH, Changes in serum chemical values as a result of prolonged contact with the clot. *Am J Clin Path* 1976; 6: 598-604.
7. Donnelly JG, Soldin SJ, Nealon DA, Hicks JM. Stability of twenty-five analysts in human serum at 22^oC, 4^oC and -20^oC. *Paediatr Pathol Lab Med* 1995; 15(6):869-74.
8. Heins M, Heil W withold W, Storage of serum or whole blood samples? Effects of time and temperature on 22 serum analytes. *Eur J Clin Chem Clin Biochem*, 1995; 33: 231-38.
9. Datta RR, Baruah A, Pathak MS, Barman M, Borah MB. Effect of temperature and serum clot contact time on the clinical chemistry Laboratory results. *Ijbamr Dec* 2014; 4(1):356-362.
10. Baruah A, Goyal P, Simha S, Ramesh KL, Datta RR. Delay in specimen processing major source of preanalytical variation in serum electrolytes.
11. Zhang DJ, Elswik RK, Miller WG, Bailey JL. Effect of serum clot contact time on clinical chemistry laboratory results. *AM J Clin Chemistry* 1998; 44: 1325-1333.
12. Gowenlock AH. *Practical clinical biochemistry.* Heinemann Medical Books, 1988:314.