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EFFECT OF SERUM SAMPLE STORAGE ON CREATININE & POTASSIUM – A STUDY IN A TERTIARY CARE CENTER

KEY WORDS: Serum, biochemical parameters, potassium

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ABSTRACT

Background: Serum samples storage for prolonged periods has been shown to alter routine biochemical parameters.
Aim: To study was undertaken to observe the levels of serum creatinine when they are stored for prolonged periods at -20°C.
Results: Significant changes were observed in serum creatinine levels after 72 hours of storage which further deteriorated when serum creatinine was estimated after 3 months.
Conclusions: Creatinine & Potassium should be estimated within 24 hours if storage is unavoidable. Biochemical parameters should be estimated as soon as possible to avoid erroneous results & better patient care.

INTRODUCTION

Biochemical estimations aid in the diagnosis and guiding treatment of various diseases. Time from collection to estimation is an important factor that can affect the reported value of an analyte. Sample storage may lead to change in physical properties because of storage conditions. These changes are also known as storage lesions of the sample (1). Commonest cause of storage lesions is hemolysis which effects the sample in number of ways. Hemolysis can affect a blood sample by erythrocyte rupture leading to release of intracellular analytes into the serum causing hemodilution or hemoglobin concentration may directly affect the particular parameter of interest (2). For routine assays in a clinical laboratory, serum is the sample. To get the real pathological picture storage lesions must be reduced to bare minimum so that they cease to have any impact on clinical interpretation of the results. According to standard guidelines plasma or serum should be separated within 20-30 min as soon as possible after clot formation is complete to avoid clot-induced changes in the concentration of serum analytes (3). Prolonged contact of serum with cells is a common cause of spurious test results, it should ideally be separated from cells as quickly as possible to prevent ongoing metabolism of cellular constituents which can have effect on estimated analytes (4, 5). Many investigators have studied related changes in some analytes, but the results are controversial. The present study was designed to determine the effect of storage time on the laboratory results of creatinine in pooled sera at different time intervals. In this study we tried to find out the quantitative alterations and the useful length of stored serum in different time on the laboratory results and the clinical significance of such alterations.

MATERIAL & METHODS

The study was conducted in the Dept. of Biochemistry, National Institute of TB & Respiratory Diseases, New Delhi. Sample anonymization was done & only the samples from the healthy male subjects in the laboratory were included in the study. After taking all the necessary aseptic precautions; 4 ml of blood was collected from healthy adult males about 20-30 years old male (in total 20 adult males) in red top vacutainer. The adult males were instructed to fast overnight before blood collection was completed. Samples were allowed to clot at room temperature for 20 minutes, and then centrifuged at 3500 rpm for 7 minutes. To get the pooled sera, serum was collected from all the samples in sterile container; mixed and aliquots of 1 ml each were prepared and stored at -20°C. Analysis was performed the separated sera for creatinine without delay (zero time). Furthermore, each sample was stored in the refrigerator at -20°C and estimations were performed 0, 24 hours, 48 hours, 78 hours, 1 week, 15 days, 1 month, 2 months, 3 months. Only one aliquot was used each time the analysis was performed.

Estimation of creatinine was done by modified Jaffes' method using ROCHE Hitachi H – 902 autoanalyzer. The samples showing visible hemolysis and the icteric samples were excluded from the study. Data were analyzed using standard statistical methods and results were expressed in percentage increase or decrease after analyzing at different intervals. Serum potassium was estimated using ROCHE electrolyte analyzer – 9180 (ion selective electrode analyser).

RESULTS & DISCUSSION

A pooled serum was made from the serum from the 20 male subjects and the pooled serum was analyzed. Mean age of the subjects was 25 ± 5 years. The various biochemical parameters analyzed are shown in Table 1.

Figure/Table 1: Assay values in pooled serum immediately after collection (0 hours), 24 hours & 48 hours with reference intervals

Analyte	Values obtained (0 hours)	Values obtained (24 hours)	Values obtained (48 hours)	Reference intervals
Glucose (mg/dl)	112.8	111.5	98.8	70 - 110
Total protein (g/dl)	7.30	7.27	7.25	4.5 - 7.5
Albumin (g/dl)	3.38	3.37	3.34	3.5 - 4.5
Total Bilirubin (mg/dl)	0.81	0.81	0.81	0.2 - 1
Direct Bilirubin (mg/dl)	0.3	0.3	0.29	0.1 – 0.4
AST (U/l)	30	31.2	32.1	0 - 40
ALT (U/l)	28.9	29.4	30.2	0 - 40
ALP (U/l)	101.3	101.7	102.3	35 - 129
Urea (mg/dl)	36.2	36.3	36.4	20 - 40
Creatinine (mg/dl)	0.72	0.93	1.0	0.7 - 1.4
Uric Acid (mg/dl)	4.8	4.76	4.73	3.5 - 7
Total cholesterol (mg/dl)	165	163.1	162.5	150 - 200
Sodium (mmol/l)	143	143	142	136 - 145
Potassium (mmol/l)	4.4	4.7	5.1	3.5 – 5
Chloride (mmol/l)	102	103.7	104.1	96 – 106

All the analytes were reassessed at the intervals of 24 hours, 48 hours, 78 hours, 1 week, 15 days, 1 month, 2 months, 3 months from the 0 hours.

Figure/Table 2: Changes in analyte concentration in pooled serum over time when stored at -20°C

Analyte	72 hours	1 week	15 days	1 month	2 months	3 months
Glucose (mg/dl)	92.1	86.4	84.3	80.2	75.4	69.7
Total protein (g/dl)	7.28	7.25	7.24	7.22	7.20	7.16
Albumin (g/dl)	3.35	3.33	3.30	3.26	3.25	3.23
Total Bilirubin (mg/dl)	0.80	0.78	0.77	0.75	0.72	0.7
Direct Bilirubin (mg/dl)	0.29	0.28	0.26	0.25	0.24	0.21
AST (U/l)	33.4	42.2	44.6	47.8	55.3	61.5
ALT (U/l)	32	38.1	43.5	48.7	52.3	58.4
ALP (U/l)	103.3	107.3	110.4	112.2	115.4	119
Urea (mg/dl)	36.3	36.5	36.6	36.9	37.1	38.2
Creatinine (mg/dl)	**1.2**	**1.4**	**1.5**	**1.7**	**1.9**	**2**
Uric Acid (mg/dl)	4.68	4.64	4.52	4.48	4.35	4.1
Total cholesterol (mg/dl)	162.1	161.8	160.4	158.2	156.3	155.8
Sodium (mmol/l)	**142**	**140**	**139**	**138**	**137**	**135**
Potassium (mmol/l)	**6.6**	**10.3**	**17.3**	**23.4**	**30.1**	**35.4**
Chloride (mmol/l)	104.7	105.5	107	108.2	111.5	113.2

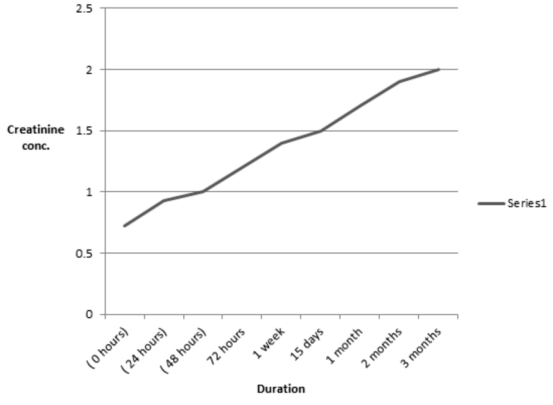
In this study we could not find any statistically significant changes in the levels of the analytes till 48 hours when the samples are stored at -20°C but significant changes are observed after 72 hours in some parameters.

As seen from the Table 2, significant changes in concentration was seen in creatinine. Creatinine concentration increased by 71.4% from the 0 hour value by the end of 72 hours and it increased by 185.7% by the end of 3 months. Potassium concentration increased by 50% from the 0 hour value by the end of 72 hours and it increased by massive 704% by the end of 3 months.

Creatinine showed increase of 71.4% after 72 hours of storage & 185% after 3 months (Figure/Table3) which might be due to interference of pseudocreatinines as shown by Heins M et al (6,7). Such massive increase cannot be explained just by haemoconcentration.

Potassium levels showed increase after 24 hours but significant increase was seen only after 72 hours. The increase in potassium after storage of centrifuged tubes is caused by the small number of erythrocytes on the surface of the polyester separation gel. Inhibition of sodium pump leads to hyperkalemia and hyponatremia as observed in present study(8,9).

Figure/Table 3: Changes in creatinine concentration in pooled serum over time



Other analytes did not show any significant alterations over a period of time when the sample is centrifuged as soon as it is received and stored under stringent storage conditions of -20°C.

Conclusions

Our study confirms that even when serum samples are centrifuged as soon as samples are received in the clinical laboratory and stored at -20°C, there will be progressive change in estimated creatinine & potassium levels after 72 hours. Our study will help to assess and interpret the creatinine as well as potassium depending upon the time of estimation and collection of serum sample in the clinical laboratory. Moreover, parameters should be assayed as early as possible after the extraction of blood sample to get valid laboratory results & prevent the misinterpretation of results.

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