Original Article

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Effect of Storage Time and Temperature on Important Biochemistry Parameters in Stored Human Blood Samples: A Cross-sectional Study

Biochemistry Section

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ABSTRACT

Introduction: Backbone of modern healthcare depends on accuracy of laboratory test results. Sometimes in busy laboratories, samples may have to be stored or transported at different temperatures and for different time periods before they could be analysed. In particularly such samples, validation of test results is very important.

Aim: To study the effect of storage time and temperature on important biochemistry analytes.

Materials and Methods: It was cross-sectional study, carried out in Department of Biochemistry at GGS Medical College, Faridkot, Punjab, India from January 2023 to December 2023. Total 40 patients' samples were first analysed immediately (0-hour samples) for important biochemical parameters i.e., glucose, urea, creatinine, uric acid, total bilirubin, direct bilirubin, Aspartate Transaminase (AST), Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), sodium, potassium, chloride, amylase, triglycerides, cholesterol, calcium, phosphorus, Creatine Kinase Myoglobin-Binding fraction (CK-MB), Lactate Dehydrogenase (LDH) and N-Acetyl Cysteine Activated Creatine Kinase (CK-NAC). Then samples were stored in two separate aliquots, to be stored at 2-8°C and at room temperature for analysis after 24-hour and 72-hour. Statistical analysis was done with Statistical Package for Social Sciences (SPSS) version 22 software. If p-value was less than 0.05, difference was considered statistically significant.

Results: At 2-8°C, mean values of blood glucose, direct bilirubin, amylase and CK-MB showed statistically highly significant decrease in 24-hour samples, and their mean values were further lower in case of 72-hour samples (all p-values <0.05). Statistically significant increase was noted in mean values of creatinine, uric acid, potassium and phosphorus in samples stored at 2-8°C for 24-hour, which were further higher in 72-hour samples (all p-values again <0.05). In samples stored at room temperature, similar pattern was seen but the changes were more significant as compared to samples stored at 2-8°C. (All p-values again <0.05). Additionally, in samples stored at room temperature, statistically significant decrease was seen in mean values of ALT, while statistically significant increase was noted in mean values of triglycerides, cholesterol, calcium and LDH, while no change was seen in mean values of potassium (p>0.05).

Conclusion: Patient samples should be tested as soon as possible to obtain the correct values, particularly for these mentioned parameters.

Keywords: Analyte stability, Biochemical analytes, Result validation, Storage period, Storage temperature

INTRODUCTION

The backbone of modern healthcare depends on accuracy of laboratory test results. Most of the clinical decisions regarding patients' diagnosis, disease progression, monitoring response to treatment and making differential diagnosis rely on them [1,2]. So undoubtedly, accuracy of all test results is utmost important. Otherwise, errors can lead to unnecessary additional investigations, increase in treatment cost or even wrong treatment of patients [3].

Many busy laboratories receive even more than one thousand samples, which may have to be stored for different time periods or transported at a particular temperature [4]. Many other factors within laboratories may also cause delay in processing samples within a stipulated period. Starting from the stage of sample collection till sample analysis, stability of all analytes must be maintained for accurate results. Stability has been defined as the time period during which an analyte value remains within established limits when sample has to be stored under a particular condition [5]. So careful monitoring of storage temperature and storage period of samples is very important for accurate results of a particular analyte in a sample [6]. Undoubtedly, validation of all test results in particularly such samples is utmost important.

India has diverse geography with different climates which makes it difficult to control temperature, particularly during transportation of samples. Additionally in many laboratories, there is limited storage space and unstable electricity supply due to which samples may be exposed to different temperatures before they are analysed [7]. Many studies have found significant effect of storage time and temperature on key serum biochemistry analytes, though many findings in cases of different parameters are debatable [6,7].

This study may guide which analytes reports may be considered valid in a human sample which had to be inevitably stored at a particular temperature and for a particular duration. This will also help making important decisions regarding storage of samples in a standardised laboratory. So, this original research study aimed to investigate the stability of all major biochemistry analytes in human serum when stored at different storage temperatures for different time periods.

MATERIALS AND METHODS

The present cross-sectional study was conducted in the Department of Biochemistry at GGS Medical College, Faridkot, Punjab, India, from January 2023 to December 2023. The study was approved by the institutional thesis and ethical committee (Ethics Committee Registration No. ECR/836/Inst/PB/2016/RR-20). The study was conducted in accordance with the Helsinki Declaration of 1975 that was revised in 2000. Informed consent was obtained from all

individuals included in this study, or their legal guardians or wards by complete explanation of the study procedure before enrolling them for the study.

Inclusion criteria: Blood samples of healthy adults who gave consent to participate in the study were collected.

Exclusion criteria:

1. Grossly haemolysed sample;
2. Grossly turbid sample;
3. Individuals who did not give consent to participate in the study.

Sample size calculation: The sample size was calculated using the two-sided paired t-test employing a significance level of 0.05 and a power of 80%. In order to detect a mean of paired differences of 0.5 with a range of estimated Standard Deviation (SD) of paired differences of 0.1 to 1 by interval of 0.1, the minimum required sample size came out to be 34. Considering a dropout/attrition rate of 10%√m nbv, the minimum required sample size becomes 38. Therefore, an overall sample size of 40 was enrolled for the purpose of this study [8].

Data Collection

Approximately, 5 mL of venous blood was collected under complete aseptic conditions in a plain vial and 1 mL sample in fluoride vial (for glucose estimation). Once the blood clotted, the tubes were kept for centrifuge at 2000 rpm for 10 minutes, and Serum and plasma were separated from venous blood samples in plain and fluoride tubes, respectively. The plasma and serum samples (0-hour samples) were immediately analysed for glucose and for all other parameters, respectively. For analysis at 24 and 72-hour, serum samples were stored at 2-8°C and at room temperature in two separate aliquots. And similarly, for estimation of glucose, plasma samples were stored at 2-8°C and at room temperature in two separate aliquots until analysed at 24 and 72-hour.

The biochemical parameters which were analysed included glucose, urea, creatinine, uric acid, total bilirubin, direct bilirubin, AST, ALT, ALP, sodium, potassium, chloride, amylase, triglyceride, cholesterol, calcium, phosphorus, CK-MB, LDH and CK-NAC. All these investigations were done on Beckman Coulter AU-480 fully automated analyser. Machine manufacturer's kits were used. All these parameters were measured and recorded in their SI units. Measurement of these analytes was based on following principles [Table/Fig-1] [9-25].

Analyte measured	Method used and reference	Cut-off range
Blood glucose	Hexokinase method [9]	Fasting 70-100 mg/dL, Random 120-140 mg/dL
Blood urea	Urease/glutamate dehydrogenase coupled enzymatic method [10]	15-45 mg/dL
Serum creatinine	Kinetic modification of Jaffe's method [11]	Adult males: 0.8-1.3 mg/dL, Adult females: 0.6-1.0 mg/dL
Uric acid	Uricase method [12]	Males: 3.5-7.7 mg/dL, Females: 2.6-6.0 mg/dL
Total bilirubin	Diazo method [13]	0.2-1.0 mg/dL
Direct bilirubin	Vandenbergh method [14]	0.1-0.3 mg/dL
Aspartate Transaminase (AST) and Alanine Transaminase (ALT)	International Federation of Clinical Chemistry (IFCC) method [15,16]	AST: 5-40 U/L ALT: 5-35 U/L
Alkaline Phosphatase (ALP)	p-Nitrophenyl Phosphate (pNPP) method [17]	Children< 18 y: 48-232 U/L, Adults: 18-63 U/L
Sodium, potassium and chloride	Ion Selective Electrode (ISE) method [18]	Sodium: 136-144 mEq/L Potassium: 3.6-5.1 mEq/L Chloride: 101-111 mEq/L
Amylase	2-chloro-4-nitrophenyl-α-D-maltotrioside (CNPG3) method [19]	0-85 IU/L
Triglyceride	Glycerol-3-phosphate Peroxidase (GPO-POD) method [20]	80-150 mg/dL
Cholesterol	Cholesterol Oxidase Peroxidase (CHOD-POD) method [21]	150-200 mg/dL
Calcium	Arsenazo III method [22]	9-11 mg/dL
Phosphorus	Ammonium molybdate method [23]	3.5-5.5 mg/dL
CK-MB and CK-NAC	Modified IFCC kinetic method [24]	CK-MB 0-25 IU/L CK-NAC 24-185 IU/L
LDH	Dehydrogenase Kinetic (DGKC) method [25]	230-460 IU/L

[Table/Fig-1]: Reference range and methods used for analytes.

STATISTICAL ANALYSIS

The results were tabulated and statistically analysed using SPSS version 22 software. Data was expressed as Mean±2 SD. To obtain mean value for each parameter, sum of the readings was done, and the obtained value was divided by 40 (total number of observations) (for each of the 40 samples, measurement of each parameter was done once). The p-value was calculated to know the statistically significant difference between the values of different parameters by paired t-test. If p-value was less than 0.05, the difference was said to be statistically significant.

RESULTS

Mean age of study participants was 41.5±4.25 years with age range of 18 to 65 years. Male participants were 19 (47.5%), and female participants were 21 (52.5%). As shown in [Table/Fig-2], when samples were stored at 2-8°C, the mean value of blood glucose, direct bilirubin, amylase and CK-MB showed statistically highly significant decrease in 24-hour samples, and their mean values were further lower in case of 72-hour samples (p-values for these parameters were <0.05). Statistically significant increase was noted in mean values of creatinine, uric acid, potassium and phosphorus in samples stored at 2-8°C for 24-hour, and their mean values in 72-hour samples were further higher (p-values for these parameters were <0.05). So, among electrolytes, only potassium showed statistically significant change. As seen in [Table/Fig-3], the mean values of glucose, direct bilirubin, ALT, amylase and CK-MB showed statistically highly significant decrease in 24-hour samples, which were further lower in 72-hour samples (p-values for all these parameters were <0.05). Statistically significant increase was noted in mean values of creatinine, uric acid, triglycerides, cholesterol, calcium, phosphorus and LDH in samples stored at room temperature for 24-hour, and their mean value in 72-hour samples were further higher (p-values for all these seven parameters were <0.05). No significant change was seen in mean value of urea in 24-hour sample (p-value>0.05), but significant decrease was seen in its mean values in 72-hour samples (p-value<0.05).

As depicted in [Table/Fig-4], the mean values of glucose, direct bilirubin, amylase and CK-MB showed statistically highly significant decrease in 24-hour samples, which were further lower in 72-hour samples (p-values for all these parameters were <0.05) at both temperatures, but the decrease was more statistically significant at room temperature in comparison to the decrease at 2-8°C (p-values for all these parameters for both 24-hour and 72-hour samples were <0.05). The mean values of creatinine, uric acid and phosphorus in samples stored at both temperatures in both 24-hour samples showed statistically significant increase (all p-values <0.05), and their mean values in 72-hour samples were further higher (all p-values <0.05). But in case of samples stored at room temperature, the increase in their mean values was more statistically significant as compared to the increase in case of both 24-hour and 72-hour samples stored at 2-8°C (p-values in case of all these parameters were <0.05).

Analyte	Time			p-value (for 0 hour and 24-hour)*	p-value (for 0 hour and 72-hour)*	p-value (for 24-hour and 72-hour)*
	0 Hour (Mean±SD)	24-hour (2-8°C) (Mean±SD)	72-hour (2-8°C) (Mean±SD)			
Glucose (mg/dL)	106.8±17.7	104.2±16.8	98.3±21.6	0.032*	0.025*	0.030*
Urea (mg/dL)	27.15±7.86	27.38±8.31	26.62±8.09	0.830	0.206	0.612
Creatinine (mg/dL)	0.78±0.25	0.82±0.27	0.90±0.31	0.028*	0.018*	0.023*
Uric Acid (mg/dL)	4.81±1.46	4.90±1.47	5.00±1.48	0.039*	0.030*	0.043*
Total Bilirubin (mg/dL)	0.84±0.52	0.82±0.51	0.81±0.51	0.151	0.149	0.150
Direct Bilirubin (mg/dL)	0.17±0.11	0.16±0.11	0.16±0.10	0.043*	0.039*	0.044*
†AST (IU/L)	35.30±19.66	34.90±19.52	34.53±20.00	0.172	0.151	0.171
‡ALT (IU/L)	44.33±32.91	43.08±32.37	42.41±32.24	0.152	0.051	0.150
§ALP (IU/L)	88.68±28.86	88.37±28.39	88.72±28.73	0.432	0.672	0.563
Sodium (mEq/L)	138.4±4.0	139.23±3.90	139.28±4.34	0.183	0.176	0.182
Potassium (mEq/L)	4.24±0.29	4.28±0.29	4.31±0.30	0.044*	0.044*	0.039*
Chloride (mEq/L)	103.7±3.39	104.93±3.36	104.2±3.83	0.190	0.212	0.212
Amylase (IU/L)	83.55±34.57	82.13±34.67	81.18±34.49	0.032*	0.025*	0.030*
Triglyceride (mg/dL)	158.0±80.7	159.3±80.9	162.5±80.8	0.133	0.047*	0.093
Cholesterol (mg/dL)	179.5±44.3	179.8±44.9	182.0±45.1	1.000	0.045*	0.049*
Calcium (mg/dL)	9.59±0.45	9.65±0.39	9.71±0.52	0.09	0.041*	0.08
Phosphorus (mg/dL)	3.63±0.42	3.71±0.42	3.76±0.43	0.047*	0.039*	0.042*
‖CK-MB (IU/L)	18.65±8.97	17.30±8.51	15.82±7.66	0.049*	0.042*	0.044*
**LDH (IU/L)	178.05±34.3	177.91±36.1	182.62±33.5	0.066	0.041*	0.05*
††CK-NAC (IU/L)	146.3±110.1	145.5±109.9	145.1±110.6	0.089	0.083	0.09

[Table/Fig-2]: Changes in the mean±SD of biochemistry parameters when samples were stored at 2-8°C for 24-hour and 72-hour.
*p-value <0.05 implies statistically significant difference; †AST: Aspartate Transaminase; ‡ALT: Alanine Transaminase; §ALP: Alkaline Phosphatase; ‖CK-MB: Creatine kinase-myoglobin binding; **LDH: Lactate Dehydrogenase; ††CK NAC: N-acetyl cysteine- activated Creatine kinase.

Analyte	Time			p-value (for 0 hour and 24-hour)*	p-value (for 0 hour and 72-hour)*	p-value (for 24-hour and 72-hour)*
	0 Hour (Mean±SD)	24-hour (RT) (Mean±SD)	72-hour (RT) (Mean±SD)			
Glucose (mg/dL)	106.83±17.77	102.75±17.26	93.57±16.61	0.028*	0.026*	0.027*
Urea (mg/dL)	27.15±7.86	27.23±8.01	24.73±7.86	0.911	0.048*	0.061
Creatinine (mg/dL)	0.78±0.25	0.84±0.29	0.94±0.34	0.021*	0.012*	0.015*
Uric Acid (mg/dL)	4.81±1.46	5.03±1.48	5.15±1.49	0.029*	0.026*	0.028*
Total Bilirubin (mg/dL)	0.84±0.52	0.82±0.51	0.78±0.51	0.151	0.140	0.146
Direct Bilirubin (mg/dL)	0.17±0.11	0.16±0.10	0.14±0.09	0.039*	0.031*	0.032*
†AST (IU/L)	35.30±19.66	33.57±19.66	32.05±18.59	0.08	0.051	0.067
‡ALT (IU/L)	44.33±32.91	42.28±31.75	39.26±28.88	0.047*	0.022*	0.035*
§ALP (IU/L)	88.68±28.86	88.85±28.62	89.70 ±29.36	0.856	0.109	0.110
Sodium (mEq/L)	138.48±4.06	138.80±3.83	139.55±4.65	0.248	0.133	0.200
Potassium (mEq/L)	4.24±0.29	4.26±0.28	4.26±0.31	0.063	0.060	0.061
Chloride (mEq/L)	103.79±3.39	104.40±2.72	104.24±3.56	0.235	0.501	0.500
Amylase (IU/L)	83.55±34.57	81.63±34.45	80.43±34.03	0.031*	0.024*	0.030*
Triglyceride (mg/dL)	158.02±80.76	162.17±81.64	166.86±81.17	0.043*	0.041*	0.042*
Cholesterol (mg/dL)	179.55±44.37	180.28±44.09	183.51±44.46	0.044*	0.039*	0.041*
Calcium (mg/dL)	9.59±0.45	9.70±0.44	9.78±0.44	0.040*	0.032*	0.038*
Phosphorus (mg/dL)	3.63±0.42	3.77±0.43	3.88±0.45	0.038*	0.029*	0.031*
‖CK-MB (IU/L)	18.65±8.97	15.82±7.77	13.35±7.07	0.044*	0.033*	0.038*
** LDH (IU/L)	178.05±34.33	181.85±35.01	188.05±35.64	0.043*	0.039*	0.041*
††CK-NAC (IU/L)	146.33±110.03	144.30±111.32	143.58±112.05	0.076	0.068	0.071

[Table/Fig-3]: Changes in the Mean±SD of biochemistry parameters when sample was stored at Room Temperature (RT) for 24-hour and 72-hour.
*p-value <0.05 implies statistically significant difference, †AST: Aspartate transaminase; ‡ALT: Alanine transaminase; §ALP: Alkaline phosphatase; ‖CK-MB: Creatine kinase-myoglobin binding; **LDH: Lactate dehydrogenase; ††CK NAC: N-acetyl cysteine- activated Creatine kinase

Analyte	24-hour (2-8* C) (mean±SD)	Time		p-value (for 24-hour (2-8* C) vs RT	p-value (for 72-hour (2-8* C) vs RT
		24-hour (RT) (Mean±SD)	72-hour (2-8*C) (Mean±SD	72-hour (RT) (Mean±SD)	
Glucose (mg/dL)	104.2±16.8	102.75±17.26	98.3±21.6	93.57±16.61	0.028*	0.026*
Urea (mg/dL)	27.38±8.31	27.23±8.01	26.62±8.09	24.73±7.86	0.911	0.048*
Creatinine (mg/dL)	0.82±0.27	0.84±0.29	0.90±0.31	0.94±0.34	0.021*	0.012*

Uric Acid (mg/dL)	4.90±1.47	5.03±1.48	5.00±1.48	5.15±1.49	0.029*	0.026*
Total Bilirubin (mg/dL)	0.82±0.51	0.82±0.51	0.81±0.51	0.78±0.51	0.151	0.05*
Direct Bilirubin (mg/dL)	0.16±0.11	0.16±0.10	0.16±0.10	0.14±0.09	0.039*	0.031*
†AST (IU/L)	34.90±19.52	33.57±19.66	34.53±20.00	32.05±18.59	0.08	0.051
‡ALT (IU/L)	43.08±32.37	42.28±31.75	42.41±32.24	39.26±28.88	0.047*	0.022*
§ALP (IU/L)	88.37±28.39	88.85±28.62	88.72±28.73	89.70 ±29.36	0.856	0.109
Sodium (mEq/L)	139.23±3.90	138.80±3.83	139.28±4.34	139.55±4.65	0.248	0.133
Potassium (mEq/L)	4.28±0.29	4.26±0.28	4.31±0.30	4.26±0.31	0.053	0.041*
Chloride (mEq/L)	104.93±3.36	104.40±2.72	104.2±3.83	104.24±3.56	0.235	0.501
Amylase (IU/L)	82.13±34.67	81.63±34.45	81.18±34.49	80.43±34.03	0.031*	0.024*
Triglyceride (mg/dL)	159.3±80.9	162.17±81.64	162.5±80.8	166.86±81.17	0.043*	0.041*
Cholesterol (mg/dL)	179.8±44.9	180.28±44.09	182.0±45.1	183.51±44.46	0.044*	0.039*
Calcium (mg/dL)	9.65±0.39	9.70±0.44	9.71±0.52	9.78±0.44	0.040*	0.032*
Phosphorus (mg/dL)	3.71±0.42	3.77±0.43	3.76±0.43	3.88±0.45	0.038*	0.029*
‖CK-MB (IU/L)	17.3±8.51	15.82±7.77	15.82±7.66	13.35±7.07	0.044*	0.033*
** LDH (IU/L)	177.91±36.1	181.85±35.01	182.62±33.5	188.05±35.64	0.043*	0.039*
††CK-NAC (IU/L)	145.5±109.9	144.30±111.32	145.1±110.6	143.58±112.05	0.076	0.068

[Table/Fig-4]: Comparison of changes in the Mean±SD of biochemistry parameters when samples were stored at 2-8*C and Room Temperature (RT) for 24-hour and 72-hour.
*p-value <0.05 implies statistically significant difference, †AST: Aspartate transaminase; ‡ALT: Alanine transaminase; §ALP: Alkaline phosphatase; ‖CK-MB: Creatine kinase-myoglobin binding; **LDH: Lactate dehydrogenase; ††CK NAC: N-acetyl cysteine-activated Creatine kinase; RT: means Room temperature

DISCUSSION

When there is delay in laboratory testing, the fundamental problem in clinical laboratories is the stability of an analyte. The quality of a laboratory report for a sample can therefore be highly affected by improper sample storage and transport in a tropical country like India. So, this study was conducted to make out which routine parameters are more sensitive to changes in storage temperature and duration.

In the present study, the mean values of glucose showed highly statistically significant decrease in both 24-hour and 72-hour samples at both temperatures. The rate of decrease in glucose concentration was more at room temperature as compared to 2-8°C. In a similar study by Marjani A, it was found that glucose concentration was decreased when samples were stored at both temperatures with increase in storage period. The decrease in glucose concentration during storage may be related to glucose sensitivity to temperature because residual blood cells within the sample continue to metabolise glucose by glycolysis, particularly at room temperature [26].

In the present study, a remarkable decrease was observed in the values of direct bilirubin from 0 to 24-hour and 0 to 72-hour at both temperatures. The rate of decrease in direct bilirubin concentration was more when samples were stored at room temperature as compared to 2-8°C. The cause may be photo-degradation, which is more at room temperature. Our study showed statistically significant decrease in the values of ALT in the 24-hour and 72-hour samples stored at room temperature, while in case of samples stored at 2-8°C, ALT showed statistically significant decrease only in case of 72-hour samples. A similar study by An B and Park CE found a significant decrease in ALT and AST values at both 22°C and 4°C [27]. On other hand, Dr R et al., found AST to be stable for up to 72-hour when samples were stored at 4±1°C [28].

In present study, statistically significant decrease was found in the values of amylase in 24-hour samples, which were further lower in 72-hour samples at both temperatures (2-8°C and room temperature). It showed that amylase values decreased with the increase in storage time and temperature. A similar study by Pahwa MB et al., illustrated that amylase was highly unstable in all conditions [29]. Our study showed that CK-MB values showed statistically highly significant decrease at both temperatures (2-8°C and room temperature), but decrease was more when samples were stored at room temperature.

The cause of decrease in the mean values of AST, ALT, amylase and CK-MB may be that these enzymes are heat-sensitive. They get denatured, leading to loss in functional shape, thus reducing their enzyme activity.

In this study, the mean concentration of creatinine, uric acid, phosphorus and LDH was significantly higher in both 24-hour and 72-hour samples stored at both 2-8°C and room temperature. The rate of increase in concentration of these parameters was more in samples stored at room temperature in comparison with samples stored at 2-8°C. Their concentration increased significantly with increase in both storage time and temperature (2-8°C and room temperature). A similar study by Marjani A found that creatinine and phosphorus concentration was increased in samples after 48-hour at 4±1°C and after 24-hour at 23±1°C [26]. The reason for increase in mean values of creatinine could be that due to bacterial growth, there is breakdown of other substances in the sample, leading to falsely elevated creatinine levels. On other hand, another study Dr R et al., found that creatinine concentration was stable up to 72-hour when stored at 4±1°C [28]. Studies by Cuhadar S et al., found that uric acid concentration increased with the increase in storage time, and its values were less stable when samples were stored at room temperature as compared to at 2±1°C [30]. The cause is enzymatic activity in the sample, causing breakdown of purines and subsequent release of uric acid. A study by Dirar AM et al., found significant increase in phosphorus in case of samples stored for 72-hour at 4±1°C and 23±1°C [31]. This may be due to breakdown of organic phosphate compounds within the residual celluar components, releasing phosphorus into the sample. Another similar study by Omar J et al., found that LDH values increased after two hours when samples were stored at room temperature [32]. The cause may be gradual release from damaged residual RBCs within the sample, causing apparent elevation in LDH levels.

The present study found that potassium was stable for up to 72-hour when samples were stored at room temperature. But at 2-8°C, statistically significant increase was noted in both 24-hour and 72-hour samples. A study by Pahwa MB et al., found that potassium values changed drastically with increasing storage period, and that it should be assessed within 48-hour when samples have to be stored at 4°C and 24°C [29]. Reason may be gradual leakage of potassium ions from residual cells into serum, primarily caused by disruption of Na+ - K+ pump in the cell membrane at 2-8°C.

In present study, no significant change was noticed in the mean values of triglycerides, cholesterol and calcium in 24-hour samples stored at 2-8°C, while statistically significant increase was found in 72-hour samples. But at room temperature, statistically highly significant increase was noted in their mean values in both 24-hour and 72-hour samples. A similar study by Saeed AA and Sheikh GA, found that the concentration of triglycerides and cholesterol were increased in all samples after 24-hour [33]. Serum triglycerides may increase at room temperature due to continued activity of lipases present in sample at room temperature which break down larger triglyceride molecules, leading to an apparent increase in measured triglyceride concentration. Cause of false high levels of cholesterol at room temperature could be because of the breakdown of lipoprotein particles in sample, causing release of free cholesterol. A study by Omar J et al., found that calcium showed significant differences in values when samples were stored at room temperature for two hours or more [32]. The reason could be that when samples are stored at warmer room temperature, there may be breakdown of residual cellular material and subsequent release of calcium ions into serum. So, samples for these three analytes should be stored at 2-8°C and they should be measured within 24-hour.

In the present study, we found that that urea was stable up to 72-hour when samples were stored at 2-8°C, while at room temperature its concentration decreased after 24-hour. Among the analytes studied by Wilson SS et al, urea nitrogen was one of the few analytes unaffected by storage for up to 42 days at different temperatures (-10, 4, 23-27, or 37 degrees C). The direction of change for all other analytes was dependent on temperature, storage duration, and the specific analyte itself [34]. The decrease in the mean value of urea in samples stored at room temperature may be because of bacterial growth which breaks down urea into ammonia. In the present study, values of total bilirubin illustrated no significant difference in 24-hour samples at both temperatures, while 72-hour samples showed a statistically significant decrease. A study by Omar J et al., also found that total bilirubin was stable at room temperature for up to 24-hour. [32] Another study by Kughapriya P et al., showed that when the samples were stored at 2-8°C, decrease in values of total bilirubin was observed after 24-hour [35]. The cause could be due to photo-degradation of bilirubin.

In the present study, no significant change was observed in the values of ALP in both 24-hour and 72-hour samples at both temperatures. Studies by Pahwa MB et al., and Cuhadar S et al., illustrated similar findings [29,30]. The cause may be that ALP is resistant to thermal denaturation. The present study found that the concentration of sodium and chloride were stable up to 72-hour at both temperatures. A study by Donnelly JG et al., had similar findings [36]. Serum proteins help stabilise many substances within it, contributing to the stability of electrolytes sodium and chloride in stored samples.

Limitation(s)

Sample size in our study was small due to financial constraints. It could be better if sample size were increased. The study could involve only those participants who visited the institute.

CONCLUSION(S)

The present study concluded that the parameters glucose, direct bilirubin, ALT, AST, amylase, CK-MB, creatinine, uric acid, potassium, phosphorus and LDH show highly significant changes with increase in duration of storage. The change was more significant when samples were stored at room temperature as compared to when samples were stored at 2-8°C. So, it is suggested that the patient samples should be tested as soon as possible to ensure validation of test results, particularly for these analytes. The present study shall guide in making important decisions regarding storage of samples in inevitable circumstances for different analytes. This study shall also guide in decision-making regarding validation of reports of these particular analytes in such samples.

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