Short communication

Effect of centrifugation force and time on the analysis of lactate dehydrogenase and potassium in the serum samples

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ABSTRACT

Introduction and Aim: Any imperfection that occurs during any stage of the testing process is described as laboratory error. Increasing requirements of biochemical tests, numerous patient samples and automation has forced laboratory work to be carried out at a faster speed. Few studies are shown to investigate the influence of settings of centrifugation of less than 10 minutes on the laboratory result in serum. Thus, our study was aimed to see the effect of centrifugation force and time on the analysis of lactate dehydrogenase(LDH) and potassium from serum samples.

Methodology: Samples were collected from 61 healthy volunteers. 5ml was taken in two separate BD vacutainer serum tubes. Tube 1 was centrifuged for 2000g for 10 minutes, tube 2 for 5 minutes 3000g, and analysed for LDH and potassium.

Results: A significant difference was observed between 5 min (U/L) (3000g) and 10 min (U/L) (2000g) with LDH and 5 min (mmol/l) 3000g and 10 min (mmol/l) 2000g with potassium.

Conclusion: LDH and potassium levels were found to be raised by increasing the centrifugal force to 3000g. Hence, the standard centrifugation protocol of 10 min at 2000 or 2500 rpm is to be followed to get the accurate results.

Keywords: Turnaround time; centrifugation; lactate dehydrogenase; potassium.

INTRODUCTION

Patient safety has finally received medical and public attention over the past 10 years, with the release of the Institute of Medicine (IOM) report To Err Is Human (1). Any imperfection which occurs at any stage during the process of testing, which includes ordering tests, reporting results, and also any factors influencing the quality of services of laboratory has been described as a laboratory error(2). Depending on what step of the testing process they occur in, errors are categorised by Karkalousos and Evangeloupos. In other words, errors can happen at any moment of the analytical process, including the pre-, during, and after stages (3). Errors affect the reproducibility, precision, accuracy and repeatability of the test results.

According to numerous publications published in the last few years, the pre- and post-analytical stages are more prone to errors than the analytical phase. This made laboratory specialists pay attention to this problem. With this, the general public and the medical community were made aware of patient protection. The only phase of analysis that is normally under laboratory control is the analytical phase; in contrast, the stakeholder and the laboratory are typically both responsible for the pre- and post-analytical phases (4). Both the analytical phase and the post-analytical phase of laboratory processing used new ways for reducing laboratory error. However, it has been observed that compared to test phases, the pre and post stages of analysis are a little more prone to errors (1).

With a reported prevalence of 3.3%, *in vitro* hemolysis is probably the most typical pre-analytic issue in laboratory medicine (5). Invisible hemolysis must be identified at an earlier point in the inquiry process since it is a significant contributor to misleading outcomes. Numerous biochemical tests, patient samples in abundance, and laboratory automation are all under increasing pressure to be completed at a faster rate.

Higher gravitational forces during centrifugation might generate mechanical stress that can lead to in-vitro hemolysis, which can unnecessarily increase serum analyte concentrations and interfere with several lab tests (6).

Another important known pre-analytical source of variation that can influence the results of laboratory tests in particular is centrifugation of samples. After spontaneous clotting, centrifugation of the serum sample should be done for at least 10 minutes at 1300-2500 g, according to clinical & laboratory standard institute (CLSI) guidelines and World health organization (WHO; 7). The centrifugation

specifications of Relative Centrifugal Field and Spin-time are generally supplied by manufacturers (8).

The consistency of the sample can be impacted by a delay in centrifugation, which can produce unreliable results (9). Even though manufacturers and guidelines advocate a 10-minute (or longer) centrifugal time sample, shorter centrifugation periods have been used in studies to reduce TAT (10). Any analyte with a plasma or serum concentration that is lower than that of red blood cells is likely to increase in the case of hemolysis (11). Variations in potassium (K) findings are particularly important because they may misidentify a situation as potentially life-threatening and lead doctors to modify unnecessary treatments (12).

Only few studies are shown to investigate the influence of settings of centrifugation of less than 10 minutes on the laboratory result in serum. Few studies have shown higher values for analytes like lactate dehydrogenase (LDH) and potassium (K+) after centrifugation for a longer time and force but few studies have also shown no influence of centrifugation time and force on these analytes. Thus, our study was aimed to see the effect of centrifugation force and time on the analysis of potassium and lactate dehydrogenase from serum samples.

MATERIALS AND METHODS

Source of data

The study was conducted using the samples obtained from Medical College and Hospital. Venous blood sample was collected from 61 healthy volunteers during the period September 2020 – march 2021.

Inclusion criteria

Blood was taken from healthy individuals who were willing to participate in the study, between the age group 20-60 years.

Exclusion criteria

Lipemic samples

Sample collection and processing

As shown in the Figure 1 shown below 5 ml of blood sample was collected from healthy individuals by experienced phlebotomists. During collections of samples, two samples were collected from each and every volunteer in two BD vacutainer serum tubes each containing 2.5 ml of sample, in a random order from one venepuncture, Tube 1 was centrifuged for 2000g for 10 minutes, tube 2 was centrifuged for 3000g for 5 minutes, and analysis was done for both lactate dehydrogenase and potassium. Values obtained were compared. Same procedure was carried out for all samples after obtaining written informed consent from the healthy volunteers. All serum samples were analysed by using the semi-automated analyser from Erba Mannheim, chem – 5 from Erba Diagnostics Mannhelm Germany.

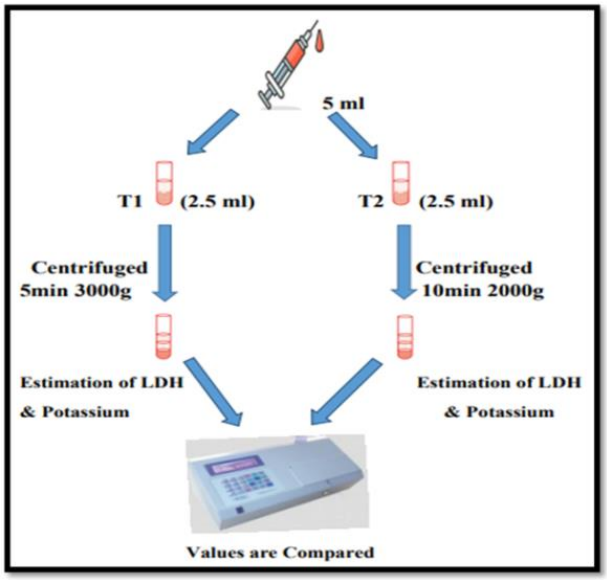

Fig. 1: Flow chart showing the method of analysis.

Method of analysis of lactate dehydrogenase and potassium (Fig. 1)

Lactate dehydrogenase was estimated using U V Kinetic method using a kit called Liqui CHEK by AGAPPE Diagnostics Kerala, India (13-15). Potassium levels were measured using selective electrode method from Roche Electrolyte Analyzer 9180: Roche Diabetes Care, Inc. Switzerland (16).

Ethical approval

The present research study involving the human participants was performed according to the ethical standards of the institutional research committee and was approved by Institutional ethics committee (MDC/DOME/369/17/07/2020).

Statistical analysis

All statistical analysis were conducted using Statistical Package for Social Sciences (SPSS V.17.0). Continuous variables were analysed by measures such as mean; standard deviation and statistical significance was tested by dependent sample t test.

RESULTS

The mean age group of the subjects whose blood was collected for estimation ranged from 21 to 36 years (Table 1).

Table 1: Age groupwise distribution

Age groups (in years)	Number	Percentage
21-25	26	42.62
26-30	17	27.87
>=31	18	29.51
Total	61	100.00
Mean	28.85	
SD	7.22	

Table 2: Values of lactate dehydrogenase (LDH) and potassium at 5 min (U/L; 3000g) and 10 min (U/L; 2000g)

Parameter	Duration	Min	Max	Mean	SD	95% CI for mean	
						Lower	Upper
Lactate Dehydrogenase	5 min (U/L) (3000g)	64.12	464.00	260.85	78.09	240.85	280.85
	10 min (U/L) (2000g)	48.09	412.00	213.14	73.18	194.40	231.89
Potassium	5 min (mmol/L) (3000g)	3.80	10.86	4.91	1.25	4.59	5.23
	10 min (mmol/L) (2000g)	3.20	10.23	4.81	1.24	4.49	5.13

Table 3: Comparison of the levels at 5 min (U/L; 3000g) and 10 min (U/L; 2000g) by dependent test

Parameter	Stages	Mean	Mean Diff.	SD Diff.	95% CI for mean Diff.		t- value	p- value
Lactate Dehydrogenase	5 min (U/L) (3000g)	260.85	47.71	43.62	36.53	58.88	8.5419	0.0001
	10 min (U/L) (2000g)	213.14						
Potassium	5 min (mmol/L) (3000g)	4.91	0.10	0.23	0.04	0.16	3.3927	0.0012
	10 min (mmol/L) (2000g)	4.81						

As shown in the tables 2 and 3, the results of the study showed a significant difference in the levels of lactate dehydrogenase (LDH) at 5 min (U/L; 3000g) and 10 min (U/L; 2000g) ($t=8.5419$, $p<0.05$) at 5% (level of significance). The mean lactate dehydrogenase (LDH) levels are significantly higher in 5 min (U/L; 3000g) as compared to 10 min (U/L; 2000g).

There was significant difference in the values of potassium, at 5 min (mmol/L; 3000g) and 10 min (mmol/L; 2000g) ($t=3.3927$, $p<0.05$) at 5% (level of significance). Thus, the mean potassium levels are significantly higher in 5 min (mmol/L; 3000g) as compared to 10 min (mmol/L; 2000g).

DISCUSSION

Improving turnaround times (TAT) is a challenging process that calls for planning, equipment, personnel training, and procurement. The entire process, from ordering the tests to reporting the results, should be tracked, and improvements should be made (17).

A study conducted by Monneret *et al.,* followed centrifugation settings of 5 min at 3000g for all immune and general chemistry, but results are varied only with LDH, and they found that increased LDH results after centrifugation for 5 min at 3000g which is in accordance with this study. The reason for increased values of LDH is due to higher g force which lead to *in vitro* hemolysis (18) which is due to increased centrifugal force (mechanical stress).

Another study conducted by Lippi *et al.,* showed that reducing time of centrifugation has no significant changes on hemolysis index, suggesting that there may be other reasons for hemolysis, which is not in agreement with our study (19).

A study conducted by Mieke *et al.,* showed no influence when the pre- analytical centrifugation time was changed between 10-15 min at 1885g. Their findings indicate that minimizing centrifugation time between 10 to 15 min at same centrifugal force (1885g) can be useful without affecting the results of the serum samples which is contradictory to our results, because the centrifugal force applied here is lesser than 2000g (20).

Limitations of the study

For further scope of the study, we can look for the hemolysis index after varying the centrifugation time and force.

CONCLUSION

The levels of lactate dehydrogenase and potassium increased by increasing the centrifugal force to 3000g in this study. The faster turnaround time met by shorter centrifugation times lead to false higher values. Hence, the standard centrifugation protocol of

10 min at 2000 or 2500 rpm is to be followed to get the accurate results of the analytes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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