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Original Research Article

Anaytical Variation Comparing Clot Separation Time and Storage For Ionized Calcium

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Abstract

To determine the variation in serum Ionized Calcium values between serum separated at 0 hour and samples when serum was separated after 4 hours with clot contact. Another comparison is between sample separated immediately and serum sample stored after separation for 24 hours at 2-8 degree refrigerated temperature. This study was done to determine the pre-analytical variation due to different storage and sample segregation methods employed by different hospitals depending upon their infrastructure and human resource. This study further emphasizes that since the variation found was minimal and non-significant in serum samples, it should be the sample of choice for the measurement if iCa. The method employed for estimation was direct ISE only and no comparison is done between the methodologies.

Keywords: Ionized Calcium, Serum Albumin.

Abbreviations

iCa: Ionized calcium
CaT: Total calcium
ISE: Ion selective electrode
PTH: Parathyroid hormone
CLSI: Clinical Laboratory Standards Institute
IFCC: International Federation of Clinical Chemistry
SPSS: Statistical Package of Social Sciences

INTRODUCTION

Ionized calcium testing has gained weightage in determining the hypocalcemia with time. Forty five to fifty percent of total calcium is only available in the free biologically active form [1-3]. Approximately 40% is protein bound and the remaining 10-15% is Ca complex to anion like sulphates, phosphates, lactate and citrate [4, 5]. We studied the serum ionized calcium time variation in random samples of the subjects in age group 18-55 years respectively. This study was mainly to determine the effect of time and storage condition on the serum ionized calcium assessed by direct ISE instrument.

One of the subcommittees of Ionized Calcium (iCa) working group was formed to gather information and formulate guidelines for collection of samples for measuring concentrations of ionized calcium; a preliminary report has recently appeared [1]. Here, we report our study of the effects of temperature and time variation of storage on results for ionized calcium, which may be useful in developing such guidelines.

We have observed little change in serum ionized calcium from serum sample analysed within 20-30 minutes, in other group of samples which was left with clot for as long as 4 hours at room temperature, which is longer than usual for processing samples for determinations of ionized calcium and 24 hour after separation from clot which was stored at 2-8 degree celcius. This study focuses mainly on the time variation of values of ionized calcium and its storage at 2-8 degree Celsius in refrigerated condition.

FoghAndersen *et al.*, [6] have also reported that clotted blood may be stored at 0-4 C for as long as 4 h without affecting results for ionized calcium. The most rapid analyses require the use of heparinized whole blood or plasma, but calcium binding by heparin and problems with protein adherence to electrodes are drawbacks to procedures involving heparin [7]. International Federartion of Clinical Chemistry (IFCC) recommends anticoagulant heparin for iCa determination [8-14]. However both sodium/lithium

heparin and calcium titrated heparin can be used. The concentration of sodium/lithium heparin should not exceed 15IU/ml and calcium titrated should not be more than 50 IU/ml blood. Heparin bind small but significant amount of calcium depending upon on quality and type of heparin used in blood. Therefore, it draws more attention in determining the procedure of analysis

In this study we wanted to determine whether lessrapid collection and handling procedures would still give satisfactory results for ionized calcium in serum.

MATERIALS AND METHOD

For the purpose of optimization of methodology for iCa determination at the department of biochemistry, Dr Ram ManoharLohia Hospital this study was undertaken from 1st December 2017 till 31st January 2018. It was given exemption from the ethical review board of our institution as it was the part of optimizing the methodology in our laboratory for patient service. Samples was collected after the consent from 40 healthy subjects. Blood sampling was done after overnight fasting under septic control with trained phlebotomist and guidelines. All the sample was collected in the prescribed vacuum containers and heparin contamination was avoided for any interference. At the time of sample collection all the subjects were made to sit, relax and breathe normally for 10 minutes. Tourniquets was applied for less than 1 minute and subjects were not allowed to exercise forearm or make fist while sampling. The samples were transported to biochemistry laboratory within 10 minutes after drawing the sample. Thereafter the samples were kept under ambient temperature of 20-25 degree Celsius until analysed. All the tubes were filled to their marked nominal value as prescribed by the vacuum container manufacturers and guidelines. All the samples were kept sealed.

Laboratory analysis

Samples was centrifuged at 3000 rpm for 5-6 min and analysed within 20-30 mins after the collection and analysed with direct ISE method, similar set of sample was left without centrifugation for 4 hours and centrifuged, serum separation and analysis was done. The serum sample was also collected and stored at 2-8 degree celcius for 24 hour and thereafter analysed for iCa. The analysis was done on Roche 9180 ISE. Assessment of variation was done between the results.

Statistical Analysis

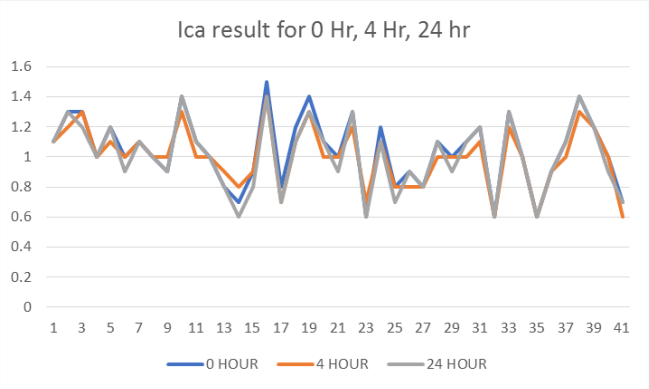
Statistical package of Social Sciences (SPSS) version 19 and Microsoft Excel was used for statistical analysis of the data. Mean and P value for the quantitative variable was computed and analysed.

RESULTS

Forty individuals participated in the study, Male with mean age 58 years and female with mean age 51 years. The other parameter for the subject was not analysed as the study focuses mainly on the temperature and clot contact time variation in the iCa.

The mean of iCa in all the three methodologies was not having significant difference where difference between 0 hour sample that is estimation of ionized calcium done 45 minutes after the sample collection including clotting time and centrifugation and after 4 hour later with the same sample in clot contact , p=1 therefore there was no significant difference in result. Other comparison was done between 0 hour sample as stated above and the sample immediately after centrifugation stored at 2-4 degree calcium in refrigerator for 24 hour was having p=1. The ionized calcium is measured in unit mmol/l.

N=40	
VARIATION	MEAN
0 HOUR	1.039
4 HOUR	1.002
24 HOUR	1.007



DISCUSSION

Ionized calcium has become more useful in clinical practice with background that total calcium concentration changes with change in albumin concentration in human body. Thus measurement of total calcium is misleading in cases with hypoalbuminemia. Of many causes of hypoalbuminemia affecting the values of total calcium are hepatic or renal diseases, burns, cardiac failure or malnutrition [15-18]. Of other cause affecting the actual value of total calcium is blood pH which alters the equilibrium constant of albumin-calcium complex, therefore acidosis reducing the binding and alkalosis increasing it [1, 7].

In this regard it became important to find out the measurement variation and clot contact time variation in samples collected for ionized calcium. The general storage as well as segregation and serum separation time of varies with different hospitals and constitutes a pre analytical variation. Our study based on this assessed the variation in serum sample when left for clot contact time for 4 hours and separated with 0 hour sample as described and serum sample stored at 2-8 degree celsius in refrigerator with 0 hour sample. Finding from this study shown that very minimal and non-significant changes in the measured values of iCa in these groups.

All the samples was assessed by direct ISE methodology and minimizing any variation with change in methodologies. This study was done on serum sample considering serum as the sample of choice for iCa estimation as serum contains no anti-coagulant to alter iCa concentration. It was also convenient the serum sample can be used for variety of test regularly.

This non significant variation allies with minimal pre-analytical variation in the serum sample for ionized calcium. This further suggests the delay in sample separation according to various setup within 4 hours accounts non-significant variation, however further time variation in clot contact is not done due to limitation of conducting the study.

CONCLUSION

In many developing countries, where various setup demands may delay the sample shifting from collection centre to laboratories performing the test and accounts for various pre-analytical variation due to delay in serum separation. This type of pre-analytical variation needs special attention and should be accounted. This study conducted assessment for ionized calcium serum separation time variation and found non-significant difference in test results between 0 hour and 4 hour sample with clot contact time and 24 hour stored sample which was separated 45 minutes after sample collection and stored at 2-8 degree Celsius in refrigerator. It has shown very minimal difference concluding that for ionized calcium. However it is always suggested that to achieve the best result sample separation should be done within 30-40 minute of sample collection.

Other variables for food, age was not considered in the study as our study was focussed clot contact time and storage variation.

Conflict of Interest: None

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