

Impedance of Results Using Lithium Heparin to Plain Tubes for Ionized Calcium

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Abstract The study was conducted to evaluate the differences in results obtained for assays of ionized calcium (iCa^{+2}) by plain and heparinised blood sample and observe for any errors in values done by ion selective electrode (ISE) method and to determine which of the collection methods could be ideal and reliable. 49 samples of heparinised and 31 plain blood samples were analyzed at lab services, Apollo Reach hospital, Karimnagar, Telangana state for iCa^{+2} by ISE method using radiometer analyzer and the differences in data were documented statistically by calculating the mean and SD. The results of the study showed statistically significant difference in values of iCa^{+2} when blood was collected in plain tube (4.7 ± 0.2) and with heparinised collection (4.4 ± 0.3). It appears in the study that plain tube collection for the assay is ideal.

Keywords: iCa^{+2} , calcium-neutralized lithium zinc heparin (CNLZ), heparinised and plain tube for iCa^{+2} , iCa^{+2} by ion selective electrode (ISE) method

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1. Introduction

Calcium is essential for many vital functions of the body. Ionized calcium represents free fraction of the total calcium in the body and is physiologically active form of calcium. In most of the laboratories total calcium is routinely measured and ionized calcium levels (iCa^{+2}) are calculated based on calcium, protein or albumin concentration. There is always a difference between calculated iCa^{+2} and the value by Ion-selective Electrode (ISE) method, but the calculated iCa^{+2} values lack precision, accuracy [1]. The measurement of iCa^{+2} levels helps in management of the critically ill patients, thus the sample for iCa^{+2} should be properly collected and measured accurately and reliably. The introduction of analyzers based on Ion-selective technology made the measurement of iCa^{+2} more reliable, rapid and reduced reporting time [2]. The sample for iCa^{+2} should be anti-coagulated with measured quantity of heparin since increased quantities of heparin may lower iCa^{+2} levels by binding with it, and the most important factor while measuring iCa^{+2} is to see that there is no change in pH of the blood [3]. Thus, handling of sample and transport of sample for analysis must be carefully done.

The present study aims to know whether there is any difference in the values of iCa^{+2} obtained from plain tube and heparinised blood tube by ISE method.

2. Materials and Methods

The study was performed at in Apollo Reach Hospital lab which included 49 blood samples from heparinised tube and 31 samples from plain tube were measured for iCa^{+2} levels by ISE method. The heparinised tubes contain Lithium heparin 75 USP units (ref. 367884). The plain samples were centrifuged at 3000 rpm and then analysed for iCa^{+2} .

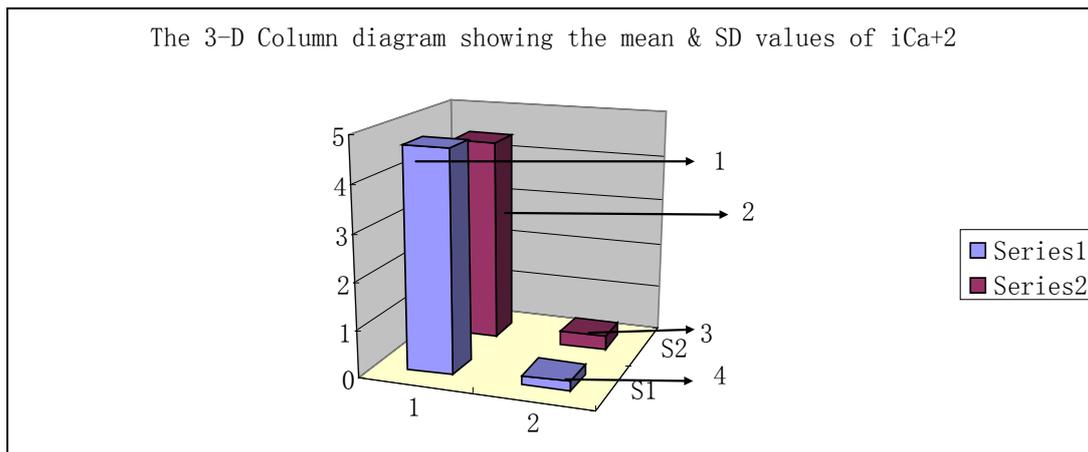
3. Results

The mean and SD values of iCa^{+2} from plain tube (4.7 ± 0.2) and that of heparinised tube (4.4 ± 0.3) were found to be significant (0.0001). (Table 1). The 95% confidence interval for iCa^{+2} from plain tube and that of heparinised tube lies in the range of 0.16 to 0.39. The 3-D column diagram of the mean and SD values of iCa^{+2} from plain and heparinised samples are shown in graph 1.

Table 1. The mean \pm SD values, p value of ionized calcium levels with heparinised and plain tubes

	(Mean \pm SD)	p value
Plain tube	4.7 ± 0.2	0.0001
Heparinised tube	4.4 ± 0.3	0.0001

The statistical analysis was done using Graph pad prism software.



Graph 1. The 3-D column diagram showing the mean & SD values of iCa^{+2} with plain and heparinised samples

- 1 – mean value of iCa^{+2} level with plain sample (without anticoagulant)
- 2 – mean value of iCa^{+2} level with heparinised sample
- 3 – SD value of iCa^{+2} level with plain sample (without anticoagulant)
- 4 – SD value of iCa^{+2} level with heparinised sample.

4. Discussion

The measurement of ionized calcium gains significance in cases where there is incomplete total calcium status such as premature infants and in individuals with disturbed plasma protein metabolism [4,5,6]. The assay of calcium is affected by many factors like pH, transport of the sample, quantity of anticoagulant used during sampling and physiological factors like postural changes [7]. An increase in pH raises ionized calcium levels and a delay in transport of sample for analysis causes build up of acid decreasing iCa^{+2} levels [8]. Change in posture causes a shift in fluid including albumin within 10 minutes to vascular compartment causing a decrease in total calcium. This may be one of the reasons for inappropriate value obtained by calculation method for iCa^{+2} . Heparin is the most common anticoagulant used for the measurement of iCa^{+2} , blood gas analysis and electrolyte levels in the blood sample [9]. The use of heparin as anticoagulant for iCa^{+2} leads to a decrease in iCa^{+2} levels in the sample due to heterogenous binding of heparin with divalent cations like Ca^{+2} [10]. Several types of heparin containing syringes are developed to minimize the interference by reducing heparin concentration absolutely necessary for anti coagulation and by using zinc salt of heparin [11,12]. Drawback of this method is that heparin binds to calcium decreasing iCa^{+2} levels. Recently developed calcium-neutralized lithium zinc heparin (CNLZ heparin) is efficient in maintaining iCa^{+2} levels in the blood sample. Here lithium binds with low affinity binding site and zinc binds with high affinity binding site of heparin and thus there is no interference of heparin on iCa^{+2} [13,14]. The ideal syringe should contain sufficient amount of anticoagulant which do not interfere with iCa^{+2} levels and that blood drawn from this syringe should be also specified for its volume. The increased/decreased blood volume for CNLZ heparin may leads to an increase or decrease in iCa^{+2} levels. In our study although we used CNLZ heparinised tube to reduce the interference of heparin, we still found that there is a decline in iCa^{+2} in most of the cases. It is not clear whether this decline in

iCa^{+2} is due to error in collecting specified quantity of the sample. Other factors including a prior pathological condition of the patient and a time delay in processing the sample may influence the iCa^{+2} levels. From the results of the study we found that among heparinised samples there is a decrease in iCa^{+2} levels when compared to the plain sample. Previous studies have also noted that use of CNLZ heparin with reduced blood volume decreases iCa^{+2} levels [15,16].

5. Conclusion

The impounding factor in the measurement of iCa^{+2} in heparin (CNLZ) collection tubes are recently developed. Ca-neutralized lithium zinc heparin method of blood collection was recommended to minimize the discrepancies in iCa^{+2} levels, but in practicality this method also noted indifferent results. These observations on heparin and plain collections showed that CNLZ heparin method can be used for iCa^{+2} measurement when a specified quantity of blood is collected, since reduced blood volume can decrease iCa^{+2} levels. Further studies on large scale are warranted to conclusively evaluate use of plain, heparinized and Ca-neutralized lithium zinc heparin blood samples for estimation of iCa^{+2} levels.

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