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Original Research Article

EDTA contamination: a preanalytical cause for interference in iron and unsaturated iron binding capacity assay

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ABSTRACT

Background: The aim of the study is focussed very keenly at performing RCA (root cause analysis) of those particular sample containing the absurd results of the Serum UIBC (Unsaturated Iron Binding Capacity), which was also tallied vigilantly side by side with Serum Iron observed at Clinical Biochemistry laboratory of The New Civil Hospital Surat, Gujarat.
Methods: The Absurd value from the samples requested for Serum Iiron and Serum Serum UIBC (Unsaturated Iron Binding Capacity) from month of August 2018 till the month of August 2019 were taken in to account for analysis. RCA (root cause analysis) of absurd value for Serum Iron and Serum UIBC (Unsaturated Iron Binding Capacity) which were prepared was mainly focused on tallying with Serum Calcium and Serum Potassium result.
Results: With the continious and strenous monitoring from the side of the researchers, the researchers had throughly analysed and found that in almost all of those analysed test containing absurd results of Serum Iron and Serum UIBC(Unsaturated Iron Binding Capacity) which were some way or the other, associated with absurd results of Serum Calcium along with absurd result of Serum Potassium too from the same samples.
Conclusions: The Absurd results of Serum Calcium and Serum Potassium are caused mainly due to pre-analytical errors more likely due to the sample contamination with EDTA (ethylene diamine tetra acetic acid). The contaminated EDTA (ethylene diamine tetra acetic acid) sample cause interference in Serum Iron measurement by producing turbidity in sample and in Serum UIBC (Unsaturated Iron Binding Capacity) by chelating Iron.

Keywords: Absurd results, Calcium, Iron, Interference, Preanalytical cause

INTRODUCTION

Interferences with clinical chemistry laboratory tests can create discrepancies in test results which can lead to patient harm. In clinical chemistry, interference is defined as a cause of medically significant difference in the measurand test result due to another component or property of the specimen. Although performance of clinical chemistry laboratory is monitored by internal QC and external quality assessment procedures, laboratories can't easily detect error caused by interferents in specimen. Interferents may originate from endogenous and exogenous source like metabolites produced in pathological conditions, compounds introduced during patient treatment like drugs, substances ingested by the patient like alcohol or nutritional supplements, substances added during specimen preparation such as anticoagulants (EDTA), contaminants inadvertently introduced during specimen handling etc.

Potassium ethylene diamine tetra acetic acid (KEDTA) is commonly used as anticoagulants in diagnostic laboratories. Markedly elevated hyperkalemia and hypocalcemia, manifests a grave potassium EDTA contamination of blood samples.[1-3] It was also a very well-known fact that calcium is chelated by EDTA, means calcium is just washed off by EDTA. False high potassium (Hyperkalemia) is often caused by pernicious

Potassium EDTA contamination and is unrecognized though very common. Moreover, Hypomagnesemia can also be caused by Potassium EDTA contamination which may leads to false result and adversely affect patient care and cost for testing.[4,5] Absurd Value are not feasible in living person. Hypocalcemia, hypomagnesemia and hyperkalemia with absurd values caused by potassium EDTA contamination is very common. It may adversely cost patient's health and waste medical resources.[5,6] Potassium EDTA easily gets detected by hyperkalemia and hypocalcemia if measured, with hypomagnesemia and hypozincemia.[7]

UIBC measurements in conjunction with serum iron measurements are used in the diagnosis and treatment of hereditary hemochromatosis and other iron disorders. The combined value of UIBC and serum iron gives a value for the TIBC. This represents the maximum concentration of iron that serum proteins can bind. Hemolysis give negative interference in UIBC measurement, so always proper blood collection technique is therefore essential to avoiding EDTA sample contamination and transfer of blood between sample tubes should always be avoided.[8] Contamination from the potassium EDTA is easily detected by marked hyperkalemia and hypocalcemia with, if measured, hypomagnesemia and hypozincemia. Surrogate markers of the EDTA contamination, like hypocalcaemia, hypozincamia, hypophosphatasia and hypomagnesaemia forfeites hyperkalaemia due to The EDTA contamination.[9] In-vitro potassium EDTA contamination due to the incorrect order of draw may depend on the type of closed venesection system.[10]

Authors had encountered absurd value of UIBC (Very High) and abnormal Serum Iron (Very High) with absurd value of Serum calcium (Very Low) and serum potassium (Very High). To understand correlation among absurd values of serum UIBC, Iron, K and Calcium this study was planned. Spurious hyperkalaemia, hypocalcaemia and hypomagnesaemia caused by potassium EDTA contamination in our studies are relatively common, and if unrecognised may adversely affect patient care and waste scarce healthcare resources.[11]

METHODS

Study was performed in the premise of clinical chemistry laboratory, New civil Hospital Laboratory Services, New Civil Hospital, Surat, Gujarat, India.

The research team in their laboratory had screened High Serum Unsaturated Iron Binding Capacity (UIBC) results from Laboratory information system over a period of one year (starting from August 2018 to August 2019).

Inclusion criteria

All high absurd results for High Serum Unsaturated Iron Binding Capacity (UIBC) (>500ug/dl) were found out and scrutinized for other allied parameters like serum iron, serum calcium and serum potassium level from same sample to check and evaluate interference. (Since there is a big chance of interference due to contamination from other test parametres, so the test is run separately in a different batch. This helps in minimizing the levels of Serum Iron and UIBC contaminations and let the team have proper, unadulterated results.

Exclusion criteria

Serum High Unsaturated Iron Binding Capacity(UIBC) results were analysed in the Auto analyser machine (ERBAXL-640) and compared with other parameters of high Serum calcium and normal Serum potassium level with proper attention and will.

All above tests were performed in Erba XL - 640 with proper vigilance and attention. Serum Calcium was measured by 'Arsenazo III ' method. Along with it, the researchers had taken Serum Potassium which was measured by 'DIRECT ISE' method. For Serum iron, it was measured by colorimetric method without precipitation. For Serum High Unsaturated Iron Binding Capacity (UIBC), it was measured by removal of excess free iron by colorimetric method. Absurd values for Serum Calcium is <3mg%, for serum K+ is >7.5 mmol/L, Serum UIBC is >500ug/dl, and for Serum iron is >500 ug/dl taken for analysis.

Statistical analysis

Statistical analysis was done by using open source Libre-Office Cal. Average, percentage and comparison of result of parameters was done by using libre cal excel.

RESULTS

Throughout the study period, the research team had been screening of about 1645 Serum Iron and Serum UIBC (Unsaturated Iron Binding Capacity) requested samples. Among them 65 samples had an absurd value for UIBC.

Table 1: Comparison of results of calcium, potassium and iron with absurd UIBC.

Total No. of Samples with UIBC >500 ug/dl	Total No. of Samples with Calcium <3 mg% with UIBC >500 ug/dl	Total No. of Samples with K >8 mmol/L with UIBC >500ug/dl	Total No. of Samples with Iron >500 ug/dl with UIBC >500 ug/dl
65	55 (85%)	60 (90%)	65 (100%)

Same 65 samples were scrutinized for serum Iron, Serum Calcium and Serum Potassium level also. From which about 55 samples i.e, (85%) out of 65 samples were hypocalcemic (absurd for calcium) and 60 (90%) out of

65 samples were hyperkalemic (absurd for K) without any clinical co-relation (Table 1 and Table 2).

Absurd hypocalcemia, hypomagnesemia and hyperkalemia, caused by potassium EDTA contamination is very common. It may adversely cost patient's health and waste medical resources. Contamination from the potassium EDTA is easily detected by marked hyperkalemia and hypocalcemia with, if measured, hypomagnesemia and hypozincemia. Various mechanisms are involved in in-vitro EDTA contamination like Decanting of blood from EDTA containing tubes into other tubes. Syringe needle contamination when delivering blood into EDTA sample tubes before other sample tubes. Proper blood collection tprocedure's essential to avoiding EDTA sample contamination. Transfering blood between sample tubes should always be avoided. Absurd hyperkalaemia, hypocalcaemia and hypomagnesaemia caused by potassium EDTA contamination are relatively common and may adversely affect patient care and waste scarce healthcare resources.

Table 2: Comparison of results in EDTA contaminated (?) and uncontaminated samples.

	Average value of S. UIBC (ug/dl)	Average value of S. Iron (ug/dl)	Average value of S.K+(mmol/L)	Average value of S.Ca++ (mg%)
Iron/UIBC/Ca2+/K+ Absurd samples	563	1547	29	0.99
Control samples	217	85	4.2	8.3

*Note: absurd value for S. UIBC and IRON each respectively to be considered are more than 500ug/Dl.

Factitious hypocalcemia, hyperkalaemia, and hypomagnesaemia caused from very low levels of potassium EDTA contamination are very difficult to detect and which could only be assured by measurement of serum EDTA. Therefore, occult EDTA contamination of blood samples can cause patient's harm and unnecessarily waste healthcare resources and its suggested that the good clinical labs should come up with effective strategies to prevent and detect wrong lab results resulting from EDTA contamination as mainly from proper blood collection techniques.

DISCUSSION

The citations being explained serially as the markedly elevated hyperkalemia and hypocalcemia, manifests a grave potassium EDTA contamination of blood samples.[1-3] The team had been analysing all contaminated (65) samples (Table 1) which gave very high results for serum calcium and potassium results. As, per the next citation, False Hyperkalemia's often caused by pernicious Potassium EDTA contamination and is unrecognized though very common. Moreover, Hypomagnesemia can also be caused by Potassium EDTA contamination which may lead to false result and adversely affect patient care and cost for testing.[4,5]

The team in the lab had not been involved in measuring serum magnesium levels, however all contaminated (65) samples analysed for absurd and high potassium, calcium results were taken in account and an average was concluded to be as K-29 mmol/L and calcium as 0.99 mg/dl which (Table 2) seemed to support the hypothetical suspicion. Hypocalcemia, hypomagnesemia and hyperkalemia with absurd values caused by potassium EDTA contamination is very common. It may adversely cost patient's health and waste medical resources.[5,6] Since it is giving a wrong result which is easily understood as practically impossible, the medical team while diagnosing a patient would have to run the tests repatedly and then conclusions can be drawn. So, all the cost burden for the wrong test result would have to be paid by the pateint himself. From the further citations, it states that Potassium EDTA easily gets detected by hyperkalemia and hypocalcemia if measured, with hypomagnesemia and hypozincemia.[7]

In our study since the team had included only four parametres as iron, UIBC, calcium and potassium, so thats why magnesium and zinc are kept excluded from the sudy discussion and is just focussed on above four parametres mentioned in Table 2. In the further citations, Hemolysis give negative interference in UIBC measurement, so always proper blood collection technique is therefore essential to avoiding EDTA sample contamination and transfer of blood between sample tubes should always be avoided.[8] This is being explained further in FIGURE 4 and 5 which has a normal serum and a contaminated serum respectively. The normal serum gives a normal graph shown below accordingly but since the abnormal graph has already chelated iron with K-EDTA, so in alkaline medim UIBC will give very high results and addition of R2 will further decrease the OD. The next citation mentions as, Surrogate markers of the EDTA contamination, like hypocalcaemia, hypozincamia, hypophosphatasia and hypomagnesaemia forfeites hyperkalaemia due to the EDTA contamination.[9] The team also has not been analysing serum phosphate levels as it was out of the scope. The contamination, impact and its effect is already being explained earlier. In vitro potassium EDTA contamination due to the incorrect order

of draw may depend on the type of closed venesection system.[10] Caution should be taken to collect sample by drawing from the veins in correct order as haphazard order of draw will cause contaminations. Further citation states as Spurious hyperkalaemia, hypocalcaemia and hypomagnesaemia caused by potassium EDTA contamination in our studies are relatively common, and if unrecognised may adversely affect patient care and waste scarce healthcare resources.[11]

The explainations had already been given in accordance to the above citation, as EDTA chelates calcium and potassium levels. Serum magnesium levels in the study is not taken in account. In Table 2 above it was concluded that the effected or contaminated samples were constantly giving very high Potassium and very low Calcium results due to EDTA interference. EDTA contamination given false high Iron as the EDTA chelates iron. To understand the phenomenon, the reaction was observed in broad day light in glass tubes. EDTA contaminated and uncontaminated samples mixed with reagent mixture in glass test-tube is observed for turbidity as shown in Figure 1.

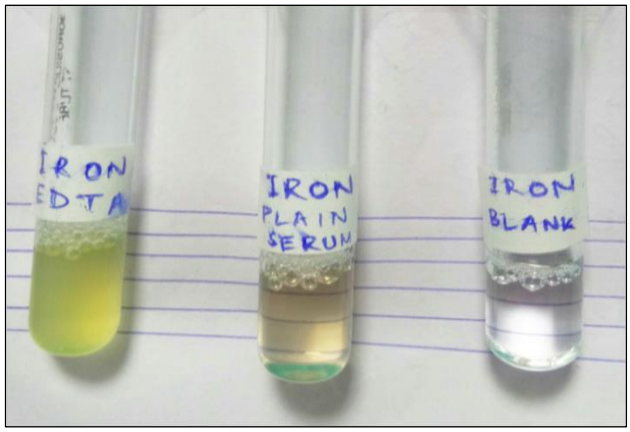

Figure 1: Comparison of iron reagents with EDTA contaminated plasma, uncontaminated serum and reagent blank.

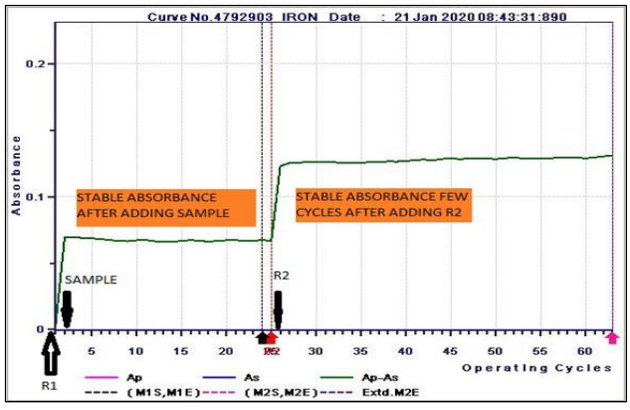

Figure 2: Reaction graph for EDTA-contaminated serum iron (*graphs from ERBA XL-640).

EDTA contamination causes incomplete precipitation of fibrinogen. Incompletely precipitated fibrinogen gets precipitated during reaction of the sample in R1 reagent of Iron having acetate buffer at pH 4.5. This is evident as very rapidly rising absorbance before addition of R2 (Figure 2). The rise in absorbance continues even after adding R2, as shown by rising absorbance (Figure 2).

Usage of surrogate markers of the EDTA contamination, like hypocalcaemia, hypozincamia, hypophosphatasia and hypomagnesaemia are of limited value detecting forfeited hyperkalaemia due to The EDTA contamination. Whereas, in the EDTA non-contaminated sample absorbance does not rise before adding the R2 (Ferrozine) (Figure 3). After adding R2 ferrozine rapidly rising its absorbance within few seconds and then after the reaction becomes a plateau. It may be very much possible that in-vitro potassium EDTA contamination due to the incorrect order of draw which may depend on the type of closed venesection system.

Above figure shows a typical normal representation of iron R1 and R2 absorbance, where as in figure 3, the optical density (od) increases abnormally with the release of R2 with constant rise of absorbance in R1.

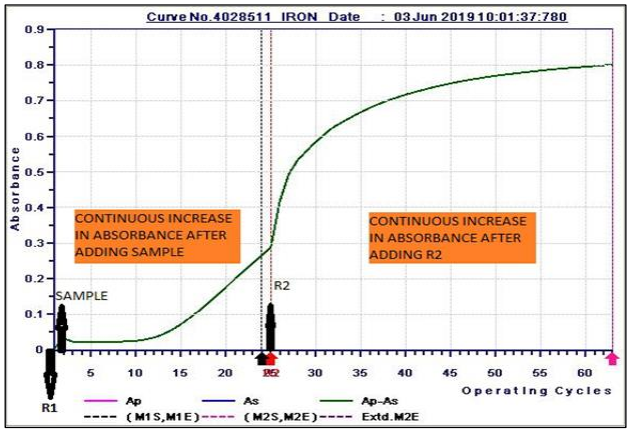

Figure 3: Reaction graph for EDTA un-contaminated serum Iron (*graphs from ERBA XL-640).

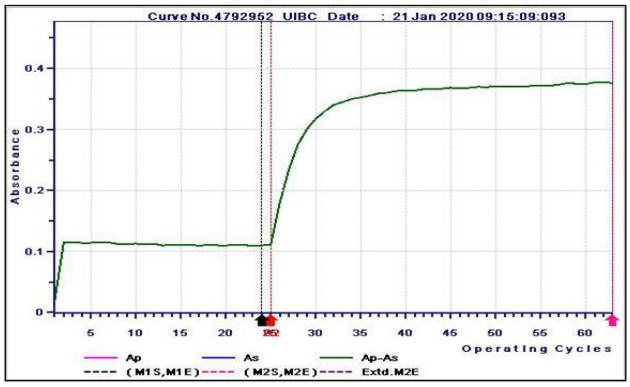

Figure 4: Reaction graph for uncontaminated serum UIBC. (*graphs from ERBA XL-640).

In UIBC assay, reagent pH is neutral (7.4) and R1 contains Ferrous ion (Fe+2) from Ferrous Nitrilo triacetic acid. When sample is contaminated by the EDTA, iron is chelated from sample as well as iron in reagent (R1) and gives low absorbance with Ferrozine (R2) resulting in very high UIBC, that might be absurd. Figure 4 and Figure 5 are comparisons of graphs for contaminated and uncontaminated samples given below.

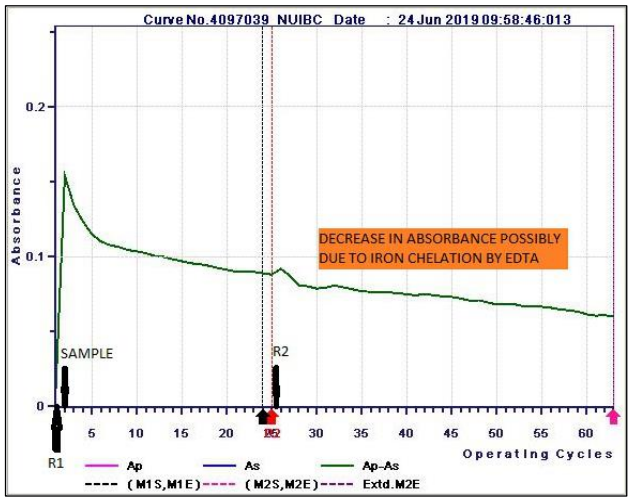

Figure 5: Reaction graph for EDTA-contaminated serum UIBC. (*graphs from ERBA XL-640).

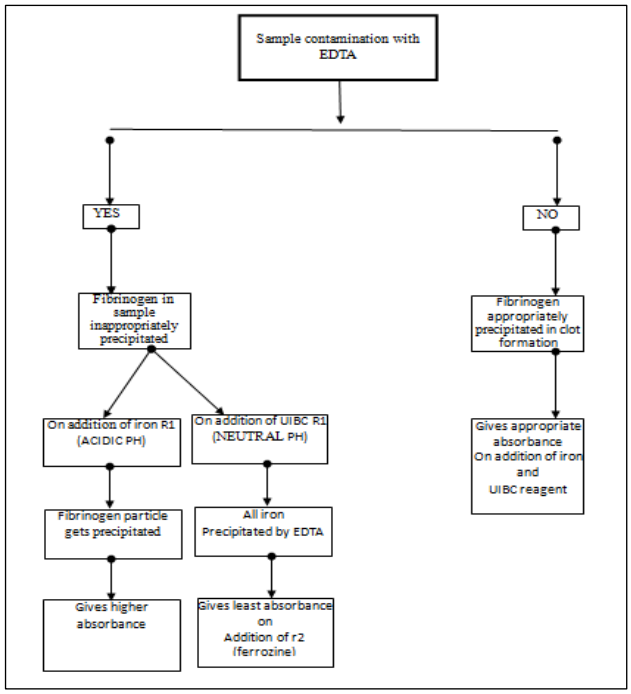

Figure 6: Phenomenon for absurd serum UIBC and iron with EDTA contamination.

The flow chart down below in Figure 6 gives a vivid summary as how the team had worked in finding the root cause analysis during the process of analysis. If the sample was found (YES) to be contaminated with EDTA, it was assumed that the sample was inappropriately precipitated so with an acidic ph of iron R1 it gave a higher absorbance and with UIBC it gave a lower absorbance.

Likewise if the sample was not contaminated (NO) with EDTA, the sample would be properly precipitated with clot formation and would give adequate absorbance both for UIBC and iron reagents.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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