

Pit falls in interpretation of electrolyte results

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Sri Lanka Journal of Diabetes, Endocrinology and Metabolism 2012; 2: 118-120

Ionized salts present in body fluids are known as electrolytes. Commonly measured human electrolytes for clinical purposes are sodium, chloride, potassium, calcium, magnesium, phosphates and bicarbonates. Electrolytes control the fluid balance in the body and are important in muscle contraction, energy generation, and almost every major biochemical reaction in the body.

Many physicians consider disease as the only cause of abnormal test results. Many factors besides diseases affect the composition of body fluids. These factors are broadly classified into 3 areas, namely pre-analytical, analytical and post analytical. Errors in interpretation of electrolyte results are encountered in certain occasions due to inadequate evaluation of pre-analytical and analytical status or conditions through which the samples are processed from the time of sample collection.

Analytic process refers to the part of the total testing process that involves measurement and analysis, as opposed to the pre-analytic part that deals with all the steps prior to performing the test and the post-analytic part that deals with all the steps after the test result is available.

There are many practices and procedures which lead to pre-analytical errors in clinical assays. These include selection of sample type (blood, serum, or plasma) appropriate to the patient, method of sample collection, site of collection (capillary, venous, arterial or proximity to IV line and conditions of sample transport and storage. Interfering substances (drugs, metabolites etc.) which are present within the sample at the time of sample analysis affect the assay procedure resulting generation of errors in the final result. Therefore it is critical to understand the impact of analytical and pre-analytical interferences in routine assays for correct interpretation.

For determination of electrolyte in serum various methods are used. The most commonly adopted methods in routine clinical laboratories include flame photometry, either direct or indirect Ion Selective Electrodes (ISE) and atomic absorption spectrophotometry.

Sodium

Pseudohyponatraemia is a state where serum sodium measurement appears low when it is actually higher than the indicated value. It is an artifact of measurement. It is

not associated with concomitant reduction in serum osmolality. There should be a high index of suspicion regarding this condition to avoid misinterpretation of results when sodium is measured using flame photometer or **indirect** ISE. Serum is composed of aqueous (water and water soluble substance) and non-aqueous (lipids and non-aqueous portion of proteins) fractions, 7% and 93% in approximate volumes respectively. Sodium is present in the serum in aqueous fraction. Lipids and proteins (non-aqueous portions) consume a portion of the volume of specimen delivered to the instrument avoiding contribution of ions for measurement. This is known as water displacement or solvent exclusion effect. This will lead to an error when the displacement occurs in abnormal proportions. The degree of error will be proportionate to the volume occupied by the non-aqueous portion of the specimen.

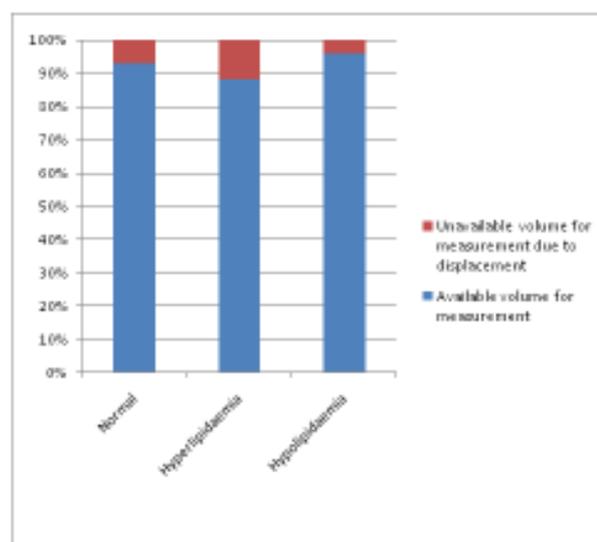


Figure 1.

In the presence of hyperlipidaemia or hyperproteinaemia, measured sodium becomes lower than the actual value when measured with above methods. In the presence of hyperlipidaemia, a physiologically normal sodium level may appear lower than the reference limit and therefore known as pseudohyponatraemia. Similarly in conditions with physiologically high levels, results can be masked,

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giving rise to normal sodium levels (pseudonormonatraemia). This pseudo effect is reversed with a decrease in the serum protein concentration resulting in pseudohypernatraemia. The fluid management based on these results that mask the true physiological condition can lead to errors resulting in serious clinical consequences. Those hyper- and hypo-pseudo effects are equally applicable to Cl⁻ as well.

These pseudo effects should be suspected in conditions such as severe hyperproteinemia (usually more than 10g/dL) and severe hyperlipidaemia (usually a triglyceride level more than 1000mg/dL) with low sodium.

Measuring serum osmolality is a suitable approach to determine whether hyponatremia represents a true hypo-osmolar state. Suspicion should also be raised when there is a discrepancy between measured osmolality and calculated osmolarity. Several formulae have been proposed to calculate serum osmolality. The most commonly used formula is Calculated Osmolality (mOsm/Kg) = 2 [Na mmol/L] + Glucose mg/dL /18 + BUN mg/dL / 2.8. In normal physiological state, the difference between measured osmolality and calculated osmolarity, the osmolar gap is approximately 12 mOsm/Kg (range 7-19).

These pseudo effects can be excluded when direct ISE is used where the activity of the relevant analyte is not affected by the concentration changes of lipids or proteins in the sample. However, a high glucose level can affect direct ISE resulting in artefactual increase in the sodium level. If direct ISE is not available, corrected sodium value can be calculated by estimating serum water fraction.

Low sodium in a patient with diabetes mellitus with poorly controlled hyperglycaemia can be a true hyponatraemia secondary to osmotic effects of hyperglycaemia. Yet this per se does not cause a hypo-osmolar state. This should be differentiated from pseudohyponatraemia secondary to associated hyperlipidaemia.

Other indirectly measured analytes such as K⁺ and Ca²⁺, are subjected to same effects as sodium but the absolute change in sodium is greater because it has a higher concentration in the serum.

Sodium values can also be affected by heparin which may be used in certain sampling devices (e.g. heparin capillary tubes used in blood gas machines) and high concentrations in samples collected through a dialysis catheter, resulting in low sodium values. The negative bias is thought to be due to chelation of Na⁺ by heparin.

Potassium

The commonest pre-analytical factor that can lead to falsely high K⁺ is haemolysis. Blood for serum electrolytes are collected into plain bottles. If by mistake blood is collected into a tube containing potassium EDTA as an anticoagulant or sodium fluoride/potassium oxalate

a blood glucose test preservative, a falsely high level of potassium or sodium or both will result due to contamination. Haemolysis should be prevented using a proper technique and usage of a correct needle with appropriate gauge and size. Haemolysis easily occurs if the blood is drawn rapidly with force to obtain samples from small gauge peripheral catheters or needles. During blood collection fist clenching should be avoided. A delay in transport of samples to the laboratory can lead to red cell break down and leakage of potassium rich intracellular fluid into the sample giving rise to falsely high values. Pseudohyperkalaemia can also occur in skin puncture derived blood (neonatal heel prick and finger prick), particularly with difficult collections. These involve excessive squeezing which leads to significant haemolysis and artefactual contamination from interstitial and intracellular fluids as well. Thus, care in sample collection for serum potassium is very important.

Occasionally falsely elevated K⁺ levels can be encountered with fragile leucocytes in patients with leukaemia, and with thrombocytosis. It is important to note that severe hypokalaemia may be masked in these conditions, giving rise to falsely high potassium levels in vitro.

Calcium

The most frequently used methods for determining plasma calcium concentration, measure total calcium, although ionized (free) calcium can be measured using ion selective electrode (ISE). Special consideration is needed in the interpretation of ionized calcium since pH changes in blood can affect ionized calcium values.

When the pH in a specimen is increased, both free calcium and free magnesium concentrations decrease due to the stronger binding of these ions with proteins in the more alkaline environments. The reverse occurs in specimens with low pH.

Gradual acidification of the sample or lowering of pH would occur, if the venous samples are left for a long period without separating serum from cells due to metabolic activity of the cells. Elevation of the pH in the specimen would result, if the venous samples are kept open and air contact is allowed, facilitating loss of CO₂ into the environment. Same consequences occur with air bubble formation during sample collection. These changes may alter the true value of ionized calcium (Ca²⁺) to a clinically significant level. Certain instruments are provided with a facility to report results to an estimated or "normalized pH", in other words to a pH value of 7.4.

Sample collection practices should be adopted to minimize these effects on ionized calcium. A half an hour rest is required prior to blood collection with no muscular action and a minimum time of tourniquet application (less than a minute) or avoiding the use of a tourniquet to prevent changes in pH and free ions binding to proteins, mainly

albumin. Exercise causes a decrease in pH due to lactic acid production and an increase in Ca^{2+} . Samples should preferably be collected into an evacuated tube containing a gel to separate serum from cells or the tube should be completely filled avoiding contact of blood with air and transported to the laboratory immediately in ice.

Similar effects can occur in ionized magnesium (Mg^{2+}) measurement, where binding of Mg^{2+} to proteins and ligands in plasma or serum is pH dependent.

When Ca^{2+} measurements are done in samples collected to heparin containing tubes, falsely low Ca^{2+} levels could be obtained. To minimize this Ca^{2+} titrated heparin tubes can be used.

Change in posture from standing to supine causes fluid shifts into the vascular compartment, decreasing albumin concentration within approximately 10 minutes. A drop of albumin by 1 g/L causes total calcium to decrease by 0.02 mmol/L leading to possible misinterpretation of results. These changes are not applicable to ionized calcium (Ca^{2+}). To prevent this, corrected calcium concentration should be used which indicates the total calcium concentration to be expected if the albumin concentration was normal (40g/L).

Sodium specifically affects plasma ionized calcium values independently to pH, presumably via modulating process of calcium binding to plasma proteins. Being aware of this fact is important in acute dilution hyponatraemia where disturbances in Na^+ concentration could alter the Ca^{2+} .

In general, erroneous results can be seen when blood is taken from catheters or cannulas which deliver IV fluids. Thus, these require flushing before collecting samples for analysis. Most frequently encountered errors occur from contamination with IV fluids containing Na^+ , K^+ , Cl^- , Ca^{2+} and Mg^{2+} . Other less common or unusual interferences occur related to lines used for infusion of drugs (e.g. ticarcillin which contains high concentrations of sodium), blood and blood products, parenteral feeds, dialysis etc. The mechanism is either additive or through binding of compounds contained in the infusions.

Therapeutic compounds can interfere with electrolyte assays by binding to electrolytes of interest or affecting the analytical procedure. Ascorbic acid (vitamin C) being a strong reducing agent, when given in high doses to cancer patients, can falsely elevate Na^+ , K^+ , Ca^{2+} and falsely reduce Cl^- levels. The activity of Ca^{2+} and Mg^{2+} ions decreases in patients receiving large volumes of blood transfusions due to chelation of these by citrate resulting in decreased measured activity.

Therefore, it is always important to interpret these results considering the clinical status of the patient and it is in fact up to the clinician to provide adequate clinical details to the pathologist for correct validation of reports.

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