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A Study on Effect of Lipemia on Electrolyte Measurement by Direct Ion selective Electrode Method

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Abstract

Background: Lipemia affects electrolyte concentration obtained by indirect ion selective electrode (ISE) method, but the specific influence on measurements by direct ion selective electrode method is yet to be clearly understood. A study was designed in this backdrop to assess possible role of escalating concentration of lipemia on electrolyte measurement by direct ISE.

Methods: Samples were collected from selected subjects in a hospital setting. A predesigned pretested format was used for recording the data from each subject. Serum sample was divided into 5 aliquots. Except for one, in the rest four, intralipid was added in escalating concentration to induce lipemia. The 5 sub-samples were tested for electrolytes and lipid concentration in parallel by two different direct ion-selective electrode methods, namely VITROS250 & HDC-Lyte. Electrolytes like sodium & potassium concentration was measured by both, while the first one also measured triglyceride concentration.

Result: Results from two instruments were compared and data were also analsyed in subgroups of standard clinical classification for sodium concentration. Considering 0-350 mg% of triglyceride as the reference, electrolytes concentration mostly decreased over increasing lipemia. Beyond triglyceride concentration of 650mg%, this decline in electrolytes concentration was statistically significantly for samples in all subgroups. In majority of samples, electrolyte values obtained from two instruments were comparable. Beyond triglyceride concentration of 1550 mg%, the sodium concentration obtained from two instruments varied significantly.

Conclusion: A correction factor may be used for the amendment of this interference property of lipemic serum samples for the major electrolytes i.e., sodium and potassium.

Keywords: Direct ISE; Lipemia; Electrolytes

Introduction

The importance of accurate measurement of electrolytes like Na+, K +, Ca++etc can never be overemphasized in terms of their critical role in clinical practice. So, measurement of the electrolytes in blood is one of the most frequently performed tests in any clinical setting. Analysis of electrolytes such as sodium and potassium are performed in the traditional clinical chemistry laboratory [1-3], but are also becoming common in point-of-care testing. Error in measurements may result in pseudohyponatremia caused by a displacement of serum water by elevated concentrations of serum lipids or proteins. Three commonly used methods for measuring sodium levels out of which two of the methods (indirect potentiometry and flame photometry) involve sample dilution and may produce falsely low sodium values thereby leading to pseudohyponatremia. This may result is so called spurious electrolyte disorders,indicating artifactually elevated or decreased serum electrolyte values that do not correspond to their actual systemic levels. The third method (direct potentiometry) involves no sample dilution, and sodium measurements are apparently unaffected by hyperlipidemia and hyperproteinemia. Direct ISE is based on a non-diluted whole-blood or plasma sample. Serum is composed of nonaqueous and water fractions, 7% and 93% of serum volume [4-8], respectively. Sodium is located in the serum water phase only. Most high-throughput laboratories use an indirect ISE, where preanalytical serum dilution is done, whereas blood gas machines make use of undiluted sample in the direct ISE. Basic process for measurement of electrolytes for both the instruments is actually same. It measures the electrolyte activity in the plasma water (mmol/kg H2O) rather than concentration in the plasma (mmol/L) [9-10]. The electrochemical activity of the ions in the water is converted to the readout concentration by a fixed (ion- specific) multiplier. Direct ion selective electrode (ISE) based result is independent of the content of solids in the sample. Studies have reported this difference of results between direct and indirect ISE. However in case of indirect ISE one study [7] performed the tests and found out that there was a marked difference in the direct ISE results and indirect ISE results. However the study was focused on the varied levels of protein concentrations of the

samples which caused the interferences in the estimation sodium levels. It did not mention about the exact concentration of protein which starts to interfere with the electrolytes measurement. Studies on the accuracy of result for electrolyte measurements by direct ISE in presence altered lipid or protein concentration are not in abundance and mostly the error in measurements in hyperlipidemia or hyperproteinemia [5] is linked to indirect method with dilution rather than that with undiluted serum. In this backdrop, we undertook the study to specifically explore the role of lipemia on electrolyte measurements by direct ISE and if it affects, then the possibility of identifying the cut-off value from where clinically significant changes ensue.

Materials and Methods

The study was undertaken in a tertiary care setting in Kolkata, Eastern region of India. The study was conceptualized by the research team and institutional approval was obtained. The study was conducted amongst subjects from inpatient departments after obtaining informed consent. Study was conducted in 2014, June-August. Initial two weeks were utilized for preparation of the study, data collection plan and case record form was designed, forms were pretested and finalized. The necessary equipment's were tested; calibrated and routine quality checks were completed. Next 10 weeks were used for data collection. A total of 128 willing subjects provided blood samples for this study. The subjects were explained about the study prior to sample collection. Standard ethical principles were followed; the study in no way interfered with case management of the subjects who agreed or refused to participate in the study.

4 ml venous blood samples were collected in a clot vial to obtain serum by Vacutainer syringe system. Samples were allowed few minutes for incubation in order to let the blood clot and to get serum. The samples were centrifuged (model REMI R-8C) at 3,500 revolutions per minute for 4 m to obtain clear serum sample. Two equipments were used for electrolytes measurements. HDC LYTE [11] (Kolkata, India) Electrolyte analyzer, which is a completely automated, microprocessor controlled electrolyte system that uses direct ISE Technology to measure electrolyte (Minimum to maximum detection limit for sodium and potassium is 40-200 mmol/L and 0.5-30 mmol/L, respectively). HDC Lyte measures various combinations of the parameters Sodium, Potassium, ionized Calcium, Lithium, pH, and Chloride from whole blood, serum, plasma, CSF, and Urine. The other one was Vitros 250 [12] (Raritan, New Jersey, USA). This uses direct ISE method for Serum, Plasma, Urine, Cerebrospinal fluid to measure electrolytes as well as lipid concentration (Minimum to maximum detection limit for sodium and potassium is 60-200 mmol/L and 1-14 mmol/L, respectively). The electrolytes measured in two instruments were compared and lipid concentration measured by Vitros-250 was documented and digitized.

Serum samples were separated from each of the clot vials with the help of micropipette into clean sterilized micro centrifuge tubes and were numbered accordingly. Then 5µl, 10µl, 15µl and 20µl of Intralipid were pipetted into four clean separate micro centrifuge tubes. INTRALIPID® 10% is a sterile fat emulsion containing soya oil, egg lecithin and glycerol. This was used to induce induce lipemia in the samples. Next, in the first two intralipid induced micro centrifuge tube i.e., 595µl and 590µl of serum from one of the previously collected and separated serum sample was added and mixed with Micropipette. After that from the first two micro centrifuge tube, 295µl and 290µl is pipette out and added respectively to the micro centrifuge tube containing 15µl and 20µl of Intralipid and mixed properly. Then , these prepared set of samples each serum sample containing a set of five; Neat, Neat+5µl, Neat+10µl, Neat+15µl and Neat+20µl] are measured in different slots in the Vitros 250 Dry Chemistry Analyzer and HDC Lyte machines simultaneously. This same procedure is carried out for the rest of the serum samples as collected. Finally, the results thus obtained from each of the instruments after completion of the measurement were digitized and analysed. Data were grouped into three categories such as normonatremia, hypernatremia and hyponatremia based on values obtained from base sample without addition of intralipid. Other than basic demographic information of the subjects, sodium, potassium concentration available from two instruments along with lipid concentration from in Vitros-250 were documented and digitized for each subject [13-15].

Statistical Analysis

Data were presented as Mean ± SD. Student unpaired two tailed 't' test and non-parametric Mann-Whitney U 't' -test was done to compare the mean of different variable. A value of $p < 0.05$ was considered as statistically significant. All statistical analysis was performed by using Graph Pad prism software (version 5, 2007, Sandiego, California, USA).

Results

All the samples were run through both the analysers, namely HDC-Lyte and Vitros-250. Results were compared for both Na+ and K+ concentrations. The summary values were obtained after categorizing the samples in three groups based on Na+ concentrations, namely normonatremic (137-145mmol/Lt), hyponatremic (<137 mmol/Lt) and hypernatremic samples (>145 mmol/Lt). Out of all 128 samples, 48 were in hypernatremic group, 40 in normo-natremic group and rest 40 in hypo-natremic group. The summary values obtained with gradual increase in triglyceride concentration were compared. In vitros-250 instrument, sodium baseline sodium concentration was 140.57 +12.97 and in HDC-lyte was 143.55 + 20.52 mmol/Lt. The figure 1 and 2 shows the change in measured electrolyte concentration i.e., sodium and potassium respectively, over increasing lipemia. Whereas, figure 3 showed a change in sodium concentration measured by two different instruments over escalating lipemia in three different categories.

The result in table 1 shows the group wise sodium and potassium concentration as available from two instruments in varying triglyceride concentration. Mean value of Na+ concentration (mmol/lt) with escalating concentration of lipid in serum samples measured by two methods of direct ion selective analysers revealed a declining trend and indicate a pseudohyponatremia with increased triglyceride concentration.

	Sodium concentration mmol/Lt		Potassium concentration mmol/Lt	
	VITROS-250	HDC-LYTE	VITROS-250	HDC-LYTE

Hyponatremic samples	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0-350	124.725	4.069823	125.9225	5.851254	4.0325	0.386561	3.982	0.433827
350-650	123.5	4.069902	123.2875	5.3246975	4.01	0.390791	3.966	0.428455
650-1050	122.45	4.063281	121.2043	5.2093855	3.985	0.383339	3.9265	0.406414
1050-1550-	121.2	4.133664	115.805	6.9069252	3.9575	0.379532	3.76125	0.430933
>1550	120.175	4.088053	110.7675	17.455091	3.9475	0.380275	3.69575	0.437187
Normo-Natremic samples								
0-350	142.25	3.550009	142.451	2.9932445	4.3875	0.382426	4.483	0.382173
350-650	140.975	3.66891	140.5787	2.455444	4.345	0.385606	4.401667	0.413289
650-1050	139.475	4.057203	138.4273	3.8520133	4.275	0.382803	4.311667	0.426575
1050-1550-	137.825	4.307938	135.8417	4.8256306	4.2375	0.380073	4.283333	0.449585
>1550	135.275	3.935294	131.3257	5.5696181	4.165	0.369719	4.118933	0.365973
Hypernatremic samples								
0-350	153.125	7.33289	160.7253	21.81612	4.7125	0.685604	4.914186	0.794164
350-650	150.4583	6.519882	151.9133	9.2148678	4.635417	0.647578	4.765116	0.689294
650-1050	148.8125	6.660957	148.9828	8.8601264	4.614583	0.658169	4.637674	0.684957
1050-1550-	147.1875	6.641764	145.9812	8.6631989	4.570833	0.643448	4.550233	0.652791
>1550	145.2083	7.130869	141.6981	7.2269859	4.529167	0.638135	4.432791	0.619736

Table 1 Concentration of electrolytes measured by two instruments with escalating concentration of induced lipemia in serum samples in three groups of baseline natremic status (n-128).

In table 2, the values obtained from two instruments were compared. Mean values obtained in different groups and from two instruments were compared with baseline triglyceride concentration of 0-350 mg/dl. This comparison showed statistically significant reduction in mean values for both the electrolytes as measured by two instruments. The intra-instrument comparison raises the concern with lipemia, as beyond 650 mg/dl concentration of triglyceride, electrolyte values changed significantly in both the instruments. Beyond triglyceride concentration of 650 mg/dl, the change in mean values obtained from baseline concentration for electrolytes were statistically significant for both sodium & potassium.

	Vitros-250			HDC-Lyte		
Sodium concenration						
Triglyceride conecntration (mg/dl)	Hypo natremia	Normal-sodium concentration	Hypernatremia	Hypo natremia	Normal-sodium concentration	Hypernatremia
0-350	Reference range for lipemia					
350-650	Sig	Sig	Sig	Sig	Sig	NS
650-1050	Sig	Sig	Sig	Sig	Sig	Sig
1050-1550	Sig	Sig	Sig	Sig	Sig	Sig
>1550	Sig	Sig	Sig	Sig	Sig	Sig
Potassiium concentration						
Triglyceride conecntration (mg/dl)	Hypo natremia	Normal-sodium concentration	Hypernatremia	Hypo natremia	Normal-sodium concentration	Hypernatremia

0-350	Reference range for lipemia					
350-650	NS	NS	Sig	Sig	Sig	NS
650-1050	Sig	Sig	Sig	Sig	Sig	Sig
1050-1550	Sig	Sig	Sig	Sig	Sig	Sig
>1550	Sig	Sig	Sig	Sig	Sig	Sig
'Sig' indicates statistically difference between two variables at the minimum level of $p < 0.05$. 'NS' indicates that there is no statistically significant difference between two variables ($p > 0.05$).						

Table 2 Statistically significant difference in values for electrolytes concentration with escalating induced lipemia in different subgroups of natremia (n-128).

Table 3 compared inter-instrument results obtained from two instruments. This showed that in majority of samples, electrolyte values obtained from two instruments were comparable. Beyond triglyceride concentration of 1550 mg%, the sodium concentration obtained from two instruments varied significantly. With gradual change in lipid concentration of the serum, results obtained for electrolyte concentration varied. Uniformly it was noted that mean values of the serum sodium concentration decreased with increase in triglyceride concentration. However the precision of the analyser-1(vitros) appears to be better compared to the HDC-Lyte, as the range of Na-mean values in serially increased concentration of triglyceride were less deviated from the baseline value in the earlier one.

Triglyceride conecntration (mg/dl)	Sodium concentration				Potassium concentration			
	Instruments	Mean (mmol/Lt)	Std. Deviation	p-value	Mean (mmol/Lt)	Std. Deviation	p-value
0-350	Vitros-250	142.25	3.55		4.39	0.382	
	HDC-Lyte	142.45	2.993	0.803	4.48	0.382	0.305
350-650	Vitros-250	140.98	3.669		4.34	0.386	
	HDC-Lyte	140.58	2.455	0.611	4.4	0.413	0.557
650-1050	Vitros-250	139.48	4.057		4.28	0.383	
	HDC-Lyte	138.43	3.852	0.279	4.31	0.427	0.707
1050-1550	Vitros-250	137.82	4.308		4.24	0.38	
	HDC-Lyte	135.84	4.826	0.075	4.28	0.45	0.646
>1550	Vitros-250	135.28	3.935		4.16	0.37	
	HDC-Lyte	131.33	5.57	0.001	4.12	0.366	0.606

Table 3 Comparison of electrolyte concentration measured by two direct ISE methods in normo-natremic subjects (n-40).
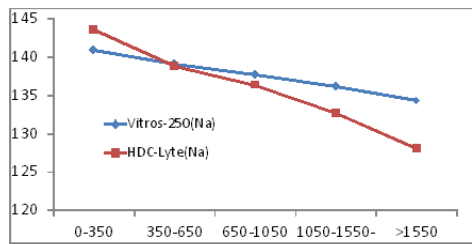
Figure 1 Line diagram showing change in measurements by two instruments of sodium concentration with changing lipemia (N-128).
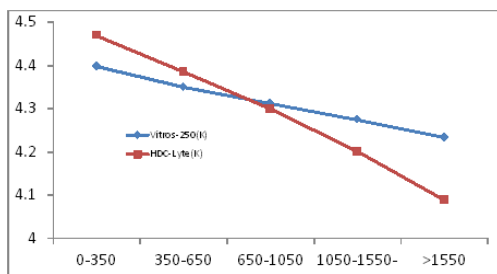

Figure 2 Line diagram showing change in measurements by two instruments of potassium concentration with changing lipemia (N-128).
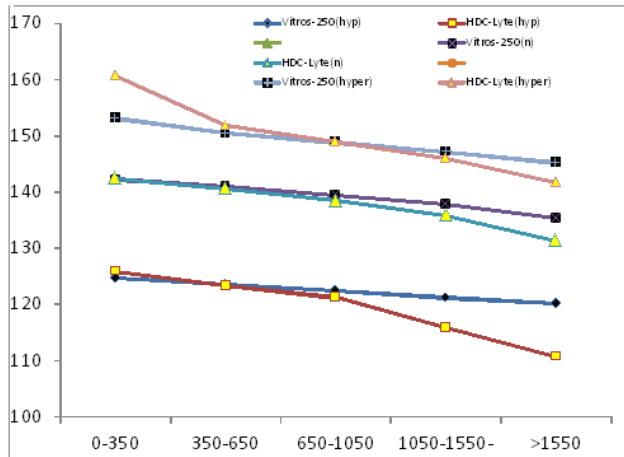

Figure 3 Line diagram showing change in sodium concentration measured by two different instruments over escalating lipemia in three different categories.

Changes of electrolyte concentration with respect to baseline values were compared with increasing lipemia. Overall trend was declining electrolyte concentration with increase in lipemia and maximum decline was noted with triglyceride concentration >1550mg/dl. In hypernatremic samples, maximum decline of sodium was 11.83% and potassium 9.79%. For normo-natremic samples sodium 7.88% and potassium 8.14%. In hyponatremic samples, sodium declined by 9.98% and potassium 7.18%. Maximum changes or decline was noted with HDC-lyte and the changes in Vitros-250 were comparatively less, though about 2.1% to 5.17% declines were noted all through.

Discussion

Our outcome showed that the concentration of sodium & potassium has decreased significantly with increased lipemia as per the maximum result obtained in normal sample and the results have gradually decreased significantly with the increase in lipemic condition. Earlier studies explored reasons behind pseudo hyponatremia but relationship with direct ISE and lipemia has never been clearly established. However, potassium is the major intracellular cation, plays a pivotal role in maintaining resting membrane potential of cells. Regulation of plasma K+ is mostly accomplished by renal excretion translocation of K+ from extracellular fluid to intracellular fluid. Marked lipemia, a condition of blood rich in emulsified fat may falsely decrease potassium due to solvent exclusion effect or volume displacement effect. Further, it has been reported that these biophysical mechanisms strongly affects concentration of electrolytes. The normal plasma consists of approximately 92% of water and 8% of lipids. In the lipemic sample, the proportion of lipid phase increases and can be up to 25%. Electrolytes that are not distributed in the lipid phase are distributed in the aqueous part of the sample, which now accounts for only 75% of the sample.13 -16

In this study, both the sodium and potassium values for the normal, hyper, and hypo conditions are found to have interference from 650 mg/dl and more so 1050-1550 mg/dl range and beyond when practically the serum almost turbid with fat. All the interference is statistically found to be significant and the maximum drift is found when the triglyceride levels are beyond 1050mg/dl. Similar studies undertaken elsewhere with indirect methods established correction factor for indirect ISE.1 The two instruments used to measure the electrolytes were mostly comparable. The tables showed no statistical difference between the results, except for that in lipemic samples beyond 1550 mg/dl. The measures of dispersion indicate the precision of vitro-250 was better compared to HDC-lyte for electrolyte measurements across the varying lipemic states.

With the results it can be concluded that direct ISE like that of indirect ISE, may influence electrolyte measurements with statistical significant drift, however to establish clinical significance may need further studies. A correction method may be justified for the amendment of this interference property of lipemic serum samples for the major electrolyte (i.e., sodium and potassium) measurements. The peranalytical dilution process is not followed in direct ISE and this may be the reason for lesser amount of change in electrolytes with changing lipemia in this method compared to that of indirect ISE.

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