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ORIGINAL RESEARCH ARTICLE

Increasing Glucose Concentrations Interfere with Estimation of Electrolytes by Indirect Ion Selective Electrode Method

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Abstract The estimation of electrolytes like sodium (Na^+), potassium (K^+) and chloride (Cl^-) using direct and indirect ion-selective electrodes (ISE) is a routine laboratory practice. Interferents like proteins, triglycerides, drugs etc. are known to affect the results. The present study was designed to look into the effect of increasing glucose concentrations on estimation of Na^+, K^+ and Cl^- by direct and indirect ISE. Pooled sera was mixed with glucose stock solution (20 g/dL) prepared in normal saline to obtain glucose concentrations ranging from ~100 to ~5000 mg/dL. Na^+, K^+ and Cl^- levels were estimated by direct and indirect ISE analyzers and results were statistically analysed using ANOVA and Pearson's correlation. Similar experiment was also performed in 24 h urine sample from healthy subjects. Significant difference was observed between Na^+ and Cl^- measurements by direct and indirect ISE, with indirect ISE values being consistently higher than direct ISE. Besides this, significant difference was observed amongst Na^+ and Cl^- values from baseline values obtained by indirect ISE at glucose concentrations ≥2486 mg/dL. However, no such difference was observed with direct ISE. Na^+ and Cl^- estimation by indirect ISE showed significant negative correlation with glucose concentration, more so, above ~2000 mg/dL. K^+, however, showed no significant difference with varying glucose. Similar results were observed in 24 h urine samples with a significant difference observed amongst Na^+ and Cl^- values at ≥2104 mg/dL glucose. Thus we conclude that high glucose concentrations interfere significantly in estimation of Na^+ and Cl^- by indirect ISE in serum as well as urine.

Keywords Direct ISE · Indirect ISE · Glucose · Electrolytes

Introduction

The measurement of serum electrolytes like sodium (Na^+), potassium (K^+) and chloride (Cl^-) is routinely performed in clinical biochemistry laboratories using ion-selective electrodes (ISE). An ISE generates a difference in electrical potential between itself and a reference electrode when the cell current is zero i.e. at equilibrium. The ISE membrane is the key component of all potentiometric ion sensors and its composition determines the optimal selectivity to the ion of interest. Specific ISE membranes can be made of glass, crystals or some specific ionophore may be incorporated in the matrix, which determines selectivity of the electrode. The membrane potential caused by selective permeability of a membrane to a particular ion and the potential generated at membrane—test solution interface is proportional to the log of ionic activity or concentration of the selected ion in solution as expressed by the Nernst equation [1]. Two methods have been described for measurement of serum electrolytes by ISE: direct and indirect. The initial methods (Indirect ISE) were based on dilution of serum with a buffer, as in flame photometry, and the

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results were comparable [2]. In the direct ISE methods the specimen is brought to the electrode surface without dilution and activity of the relevant ion is measured in the plasma water. The dilution of a sample has important implications in cases where the solid component of plasma is increased and is known as 'electrolyte exclusion effect' which often leads to falsely low values of serum electrolytes especially Na^+, recognized as 'pseudohyponatremia' [3]. This arises most commonly in situations of extreme hyperproteinemia or hyperlipidemia. Numerous methods have been employed by various authors to measure corrected Na^+, K^+ and Cl^- based on the concentration of protein, albumin or triglycerides. However, they are prone to either overestimation of fall in sodium levels or are complex calculations or do not take into account all the parameters comprising solid phase of plasma [4]. Direct ISE methods are not affected by this phenomenon as there is no dilution of the sample and moreover, whole blood can be used directly for rapid estimation as in case of open heart surgery. The electrolyte exclusion effect not only affects sodium but also other ions like potassium, chloride, magnesium, bicarbonate etc. However reduction in sodium often becomes clinically significant as it falls below the reference range due to this effect. Hence, the term pseudohyponatremia has gained more acceptance. Besides protein and triglycerides, other solutes like glucose, urea etc. might also affect the analysis of serum electrolytes by direct and indirect potentiometric methods. One such case has been reported in literature wherein the authors have shown discrepancies between the two methods with high glucose concentrations which resolved with subsequent treatment and fall in glucose levels [5]. High glucose in blood results in dilutional hyponatremia due to osmotic effect that results in hyponatremia but that occurs in vivo and is not dependant on type of method used for sodium estimation. The present study was conducted with an aim to study the effect of increasing glucose concentrations on estimation of electrolytes like Na^+, K^+, Cl^- by direct as well as indirect ISE methods.

Materials and Methods

Preparation of Samples

Randomly selected sera samples submitted for routine biochemical estimations were pooled for the study. The baseline data for the electrolytes, glucose, liver function tests, renal function tests, total protein, albumin and triglycerides were run on Modular P 800 autoanalyzer (Roche Diagnostics, Germany) equipped with indirect ISE 900 module, along with routine clinical samples after routine calibration and quality control check (Mean for QCs of Na^+, K^+ and Cl^- of 142, 6.45 and 110 mol/L respectively and coefficient of variation for QCs of Na^+, K^+ and Cl^- of 0.005, 0.017 and 0.011 % respectively). Baseline data was also generated for the electrolytes Na^+, K^+ and Cl^- on the Combiline direct ISE analyzer (Eschweiler Products, Germany) after satisfactory quality control check (Mean for QCs of Na^+, K^+ and Cl^- of 140, 4.3 and 102 mmol/L respectively and coefficient of variation for QCs of Na^+, K^+ and Cl^- of 0.004, 0.027 and 0.015 % respectively).

A stock solution of glucose with concentration of 20 g/dL was prepared by dissolving anhydrous glucose in normal saline (0.9 % NaCl). Next the pooled sera was divided into several aliquots with 3.0 ml sera each and increasing amounts of the stock solution were added to the different aliquots so as to generate glucose concentrations ranging from around 100 mg/dL to about 5000 mg/dL; volumes of the different aliquots were made up to 4 ml by adding normal saline to maintain equal dilution of the electrolytes.

A 24 h urine sample was collected from a healthy control. Glucose was then added to it to obtain glucose levels ranging from 100 to 5000 mg/dL. These aliquots were then processed for electrolyte estimation by indirect ISE.

Measurement of Analytes

Each serum aliquot was tested five times by both indirect and direct ISE analyzers and each urine aliquot was also tested for five times by indirect ISE. Glucose concentrations were measured by Modular P 800 autoanalyzer using glucose oxidase–peroxidase method (Glucose GOD-PAP kit, Roche Diagnostics, Lot no. 680760-01) in both serum and urine.

Statistical Analysis

Results are presented as mean ± SD values and two-way ANOVA with Bonferroni post-test was performed using Prism5™ software to analyse the differences between direct and indirect ISE values and also at different glucose concentrations with respect to the baseline value. Pearson's correlation was calculated using SPSS Version 16 (SPSS Inc, Chicago, IL). $p < 0.05$ was considered to be statistically significant.

Results

Baseline Biochemistry Profile

The total protein and triglyceride concentration were observed to be within the physiological range in the pooled

sera ruling out any significant interference due to these analytes (Supplementary Table 1). The glucose concentrations of the different serum aliquots obtained on Modular P were found to range from 91.3 to 4694 mg/dL. The urine showed glucose levels ranging from 0 to 5172 mg/dL.

Analysis of Electrolytes by Direct and Indirect ISE Methods

The concentrations of Na^+, K^+, Cl^-, in serum at different glucose concentrations as measured by direct and indirect ISE and those of urine by indirect ISE is shown in Figs. 1 and 2 respectively. Na^+ and Cl^- values by indirect ISE showed significantly higher values than that of direct ISE. Besides this, the serum Na^+ and Cl^- values showed significant difference ($p < 0.05$) at glucose concentrations above 2486 mg/dL from the baseline value at glucose concentration of 91.3 mg/dL by indirect ISE method. However, significant difference was not observed in case of direct ISE. No significant difference was observed between serum K^+ levels by either of the two methods at any of the glucose concentrations. Urinary Na^+ and Cl^- also showed significant difference from baseline at 2104 mg/dL glucose as depicted in Fig. 2. Interestingly, significant differences were also observed for K^+ levels, probably a reflection of higher K^+ content in urine compared to plasma/serum.

Correlation Between Direct and Indirect ISE

The Pearson correlation coefficient, R was estimated for each of the electrolytes for both direct and indirect ISE with glucose concentrations. Significant correlation was not observed in Na^+, K^+ or Cl^- levels with increasing glucose concentration by direct ISE method. However, with indirect ISE, strong and significant negative correlation was observed for Na^+ and Cl^- in serum as well as urine as shown in Figs. 3 and 4 respectively. Urinary K^+ also showed a similar trend. The Na^+ values obtained by indirect ISE were further analysed by dividing the data into two groups: one with glucose concentrations lesser than 1000 mg/dL levels, the Na^+ values showed weak and non-

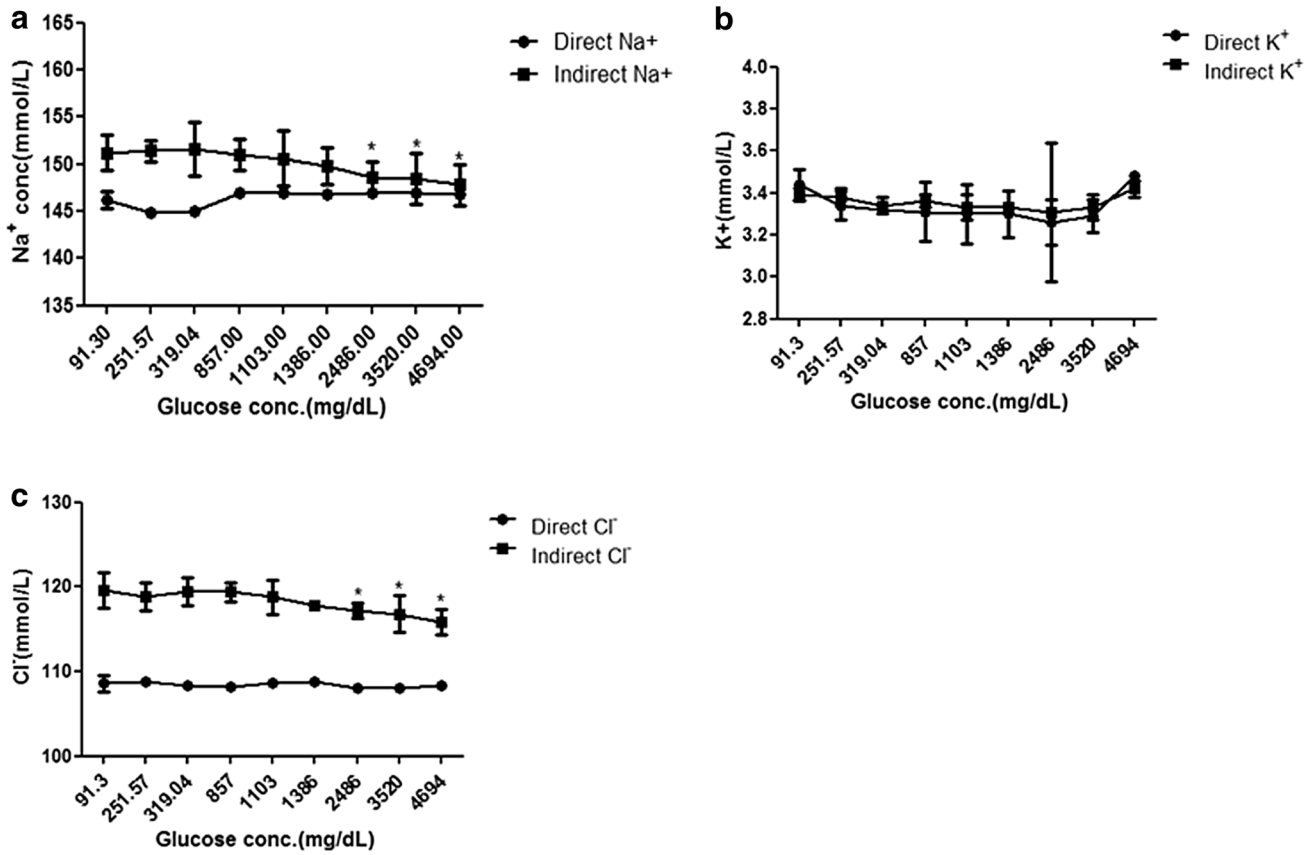

Fig. 1 Means-plot representing mean ± SD of serum electrolytes i.e. **a** Na^+ levels, **b** K^+ and **c** Cl^- at increasing glucose concentrations by direct as well as indirect ISE methods. *Signifies $p < 0.05$ by two way-ANOVA followed by Bonferroni post-tests to represent the significance of difference between each value with their respective baseline electrolyte value at glucose conc. = 91.3 mg/dL (data shown in supplementary Table 3)

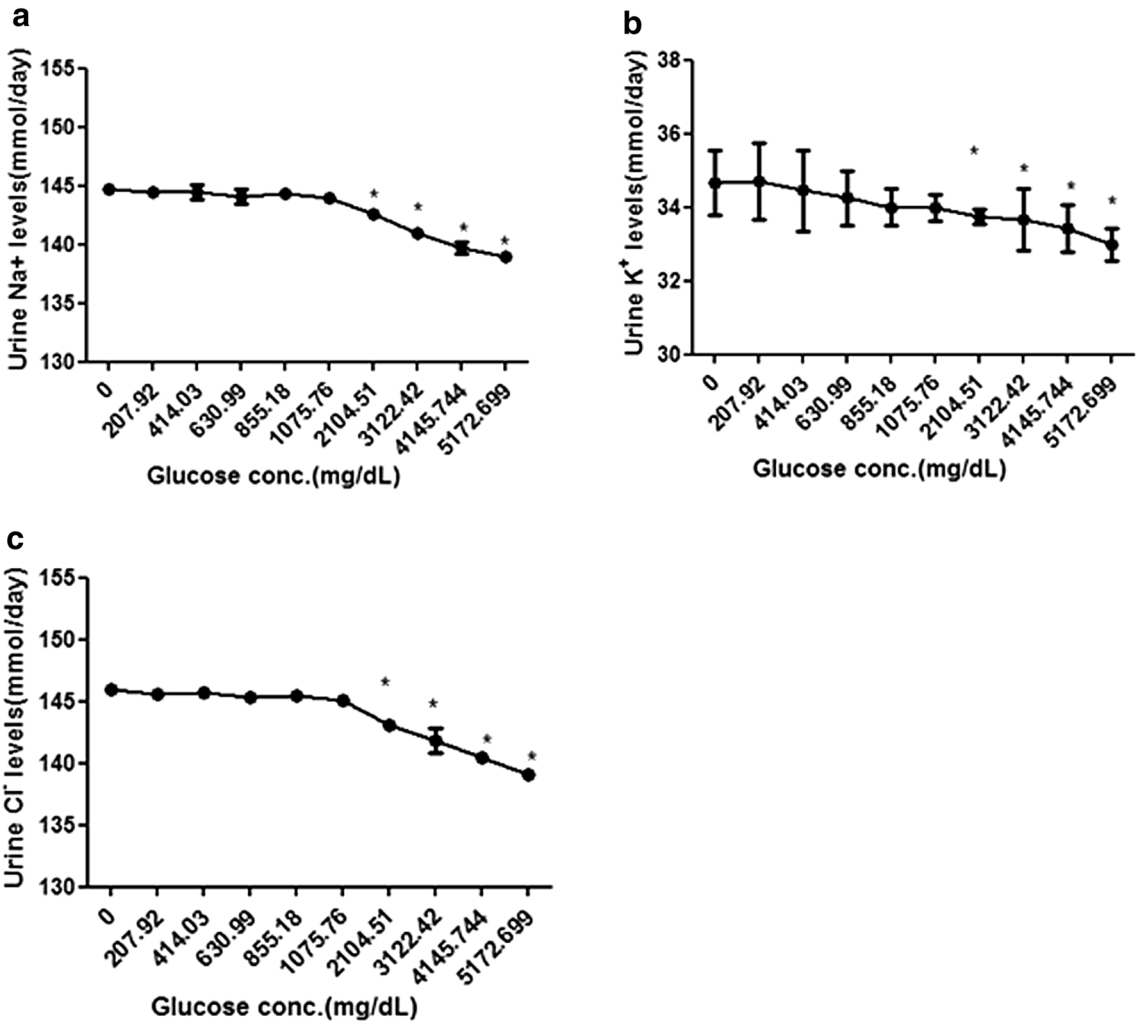

Fig. 2 Means-plot representing mean ± SD of 24-h urine electrolytes i.e. **a** Na^+ levels, **b** K^+ and **c** Cl^- at increasing glucose concentrations by indirect ISE method. *Signifies $p < 0.05$ by two way-ANOVA followed by Bonferroni post-tests to represent the significance of difference between each value with their respective baseline electrolyte value at nil glucose concentration (data shown in supplementary Table 3)

significant negative correlation (R = −0.346). However, at glucose values higher than 1000 mg/dL, the Na^+ values revealed strong and significant negative correlation (R = −0.941*). This implied that sodium values estimated by indirect ISE decreased significantly at higher glucose concentrations.

Discussion

The measurement of ions like Na^+, K^+, and Cl^- is most commonly done by electrochemistry that is based on the measurement of an electrical signal generated by a chemical system in an electrochemical cell. Ion selective electrodes use this principle of potentiometry to measure electrolytes and are routinely used in clinical laboratories for the same.

However, ISEs do not measure concentration (c); they measure only activity (a) of the ions, defined as the product of activity coefficient (γ) and concentration (a = c x γ). γ is assumed to be 1 at infinite dilution and is dependent on the concentration and valence of all the ions that are present [6]. In indirect ISE methods the sample is diluted with high ionic activity buffers in ratios of 1:20–1:34 as per the analytical systems involved [6, 7]. This ensures that the activity coefficient virtually remains constant for different samples. Thus indirect ISE yields a good estimate of concentration yielding results comparable to flame photometry. Most of the modern day autoanalyzers use this method; however they are calibrated using standard solutions with normal concentration of solids (proteins and lipids) approximately 7 % of the total plasma volume. Hence they are susceptible to 'pseudohyponatremia', a

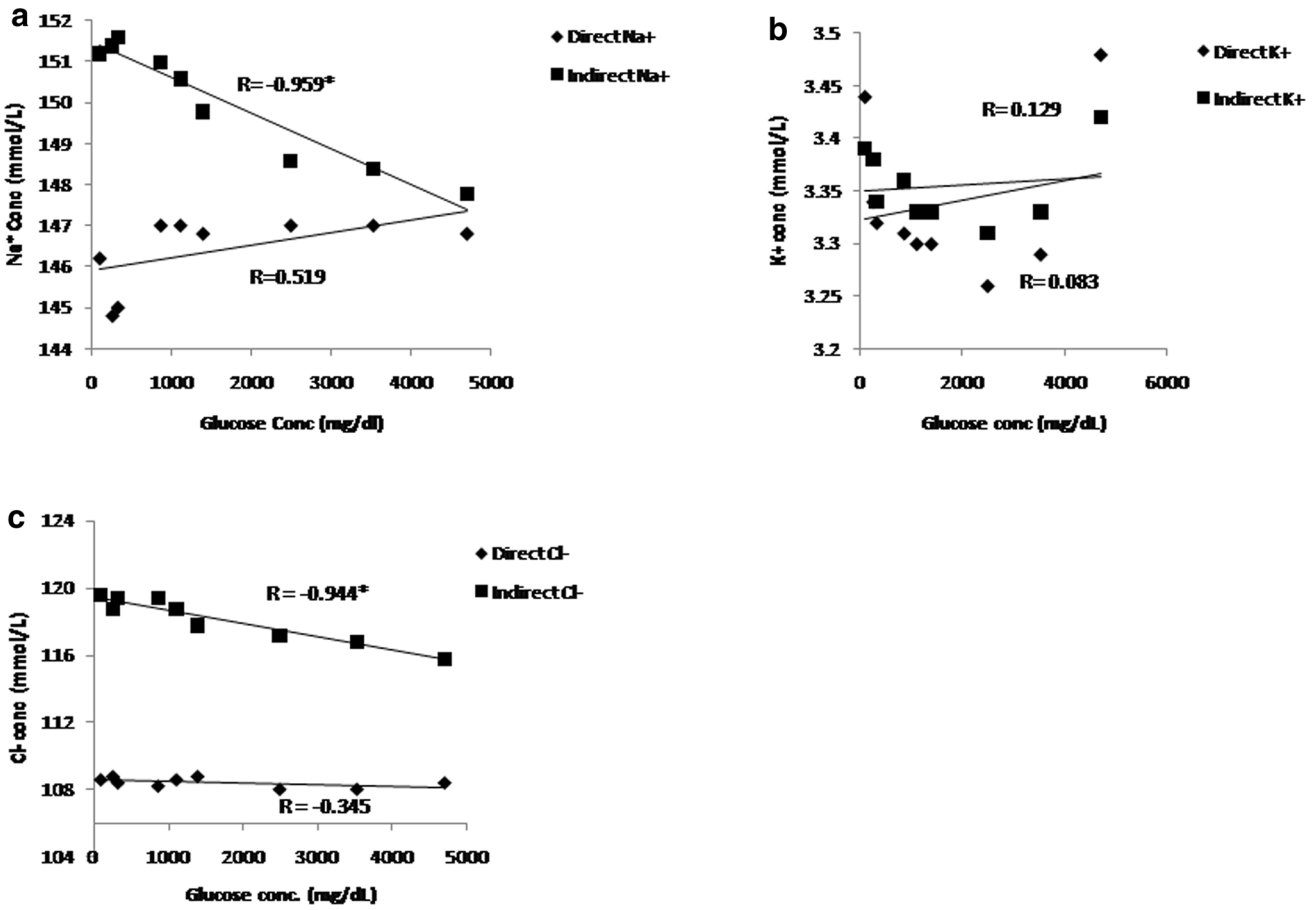

Fig. 3 Scatter plot with linear trend line representing Pearson's correlation between increasing glucose concentrations and serum electrolyte levels i.e. **a** Na^+ levels, **b** K^+ and **c** Cl^- by direct and indirect ISE methods. R represents Pearson's coefficient (extent of correlation). *Represents significant correlation at $p < 0.05$ (data shown in supplementary Table 4)

condition most evident in samples with hyperlipemia and hyperproteinemia [8]. On the other hand, direct ISE methods, where the electrode is directly exposed to undiluted sample are commonly available at the point of care for rapid determination of electrolytes in whole blood. It ensures measurement of the physiologically active fraction of the ion of interest and thus not affected by concentration of solids in the plasma (proteins and lipids). However, in biological samples like blood, plasma or serum the activity coefficient, γ would vary from sample to sample and should never reach beyond 0.7 under normal circumstances. Besides, variations due to changes in ionic strength due to other ions present has to be also accounted for [7]. Estimation of Na^+ has been reported to be affected by several factors: the type of electrode used, the amount and type of heparin used, the pH and bicarbonate level in blood to name a few [9]. Apart from the above, some studies have suggested possible interference in Na^+ estimation due to high glucose levels in the sample [5, 9]. In the present study we evaluated the effects of increasing glucose concentrations on the measurement of Na^+, K^+ and Cl^- by direct as well as indirect ISE methods.

We observed fall in Na^+ levels by indirect method with increasing glucose concentrations, which became significant at glucose concentrations $\geq$2486 mg/dL as also depicted by Pearson's correlation that showed highly significant negative correlation at glucose concentrations above 1000 mg/dL. However, we recognize the fact that these findings may not be clinically very relevant because we seldom encounter blood glucose levels beyond 1000 mg/dL. Hence, we designed a similar experiment with urine and found that results were following a similar trend as in serum above 2104 mg/dL. A similar experiment as ours has been reported in literature where the authors investigated variations in impedance values in samples of 0.9 % NaCl solution with increasing glucose concentrations from 75 to 5000 mg/dL [10]. They found that only on reaching equimolar concentration ($\sim$145 mmol or $\sim$2531 mg/dL of glucose), not in physiological range, glucose interacts with Na^+ resulting in change in

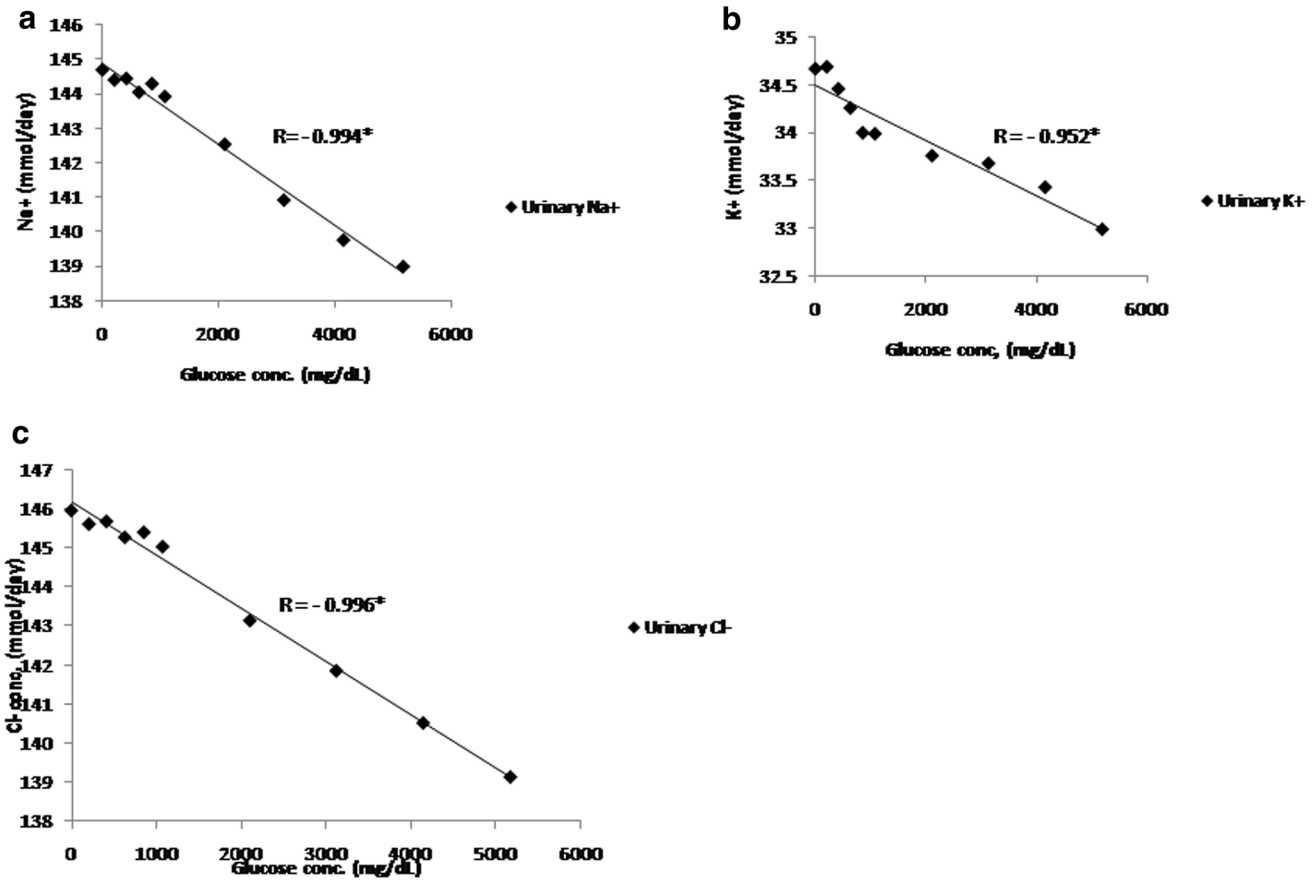

Fig. 4 Scatter plot with linear trend line representing Pearson's correlation between increasing glucose concentrations and urine electrolyte levels i.e. **a** Na^+ levels, **b** K^+ and **c** Cl^- by indirect ISE methods. R represents Pearson's coefficient (extent of correlation). *Represents significant correlation at $p < 0.05$ (data shown in supplementary Table 4)

impedance of NaCl solution [10]. This could be the plausible explanation for the observation of marked fall of Na^+ concentration by indirect ISE beyond glucose concentrations of 2486 mg/dL in the current study. However, direct ISE Na^+ measurements have not reflected the same, probably because here the samples are not diluted and hence, the activity coefficient of the ions are much lower than 1 and hence no possible interaction between sodium ion and glucose. In indirect ISE the sample is diluted so that activity coefficient increases to nearly 1 facilitating ionic interactions between glucose and Na^+ ions at various positions of glucose as evident from both quantum mechanics and molecular dynamic simulation studies [11].

It was also observed that there was a difference between baseline Na^+ and Cl^- results obtained from direct and indirect ISE even after satisfactory calibration & quality control as per manufacturers' prescribed SOPs. Na^+ and Cl^- measurement by direct ISE method in the serum shows consistently lower levels compared to indirect method that may result in difference in calculated osmolarity of the order of 2–13 mOsm/L solvent (Supplementary Table 2) and also difference in management of critically ill patients. So, different reference range should be used for different methods. Moreover, further studies with large sample size should be carried out to find out that which of the two, direct or indirect ISE methods is more suitable for estimation of sodium or chloride. However, the present study was designed to focus on interference of high glucose concentrations on ISE rather than difference between the two methods.

Our results of baseline difference are contrary to the finding of Musheifri et al. [5], where authors stated that there is no difference in baseline Na^+ values between Direct and indirect ISE. This difference at the base line plausibly due to the difference of calibration methods being used for direct and indirect methods, QC compositions as well as the difference in the composition of membranes.

However, it is clearly observed in case study by Musheifri and Jones that index case had lower sodium levels (100 mmol/L) with indirect than direct (109 mmol/L)

at high glucose concentration similar to our findings which became nearly equal ($\sim$141 mmol/L) at normal glucose concentration. Since the case is a diabetic patient, dilutional hyponatremia could be the possible mechanism for fall in the direct ISE and Indirect ISE at high glucose concentration. On the other hand, in our study pooled sera or urine was spiked with glucose in vitro and used for the experiments.

Apart from Na^+ we also analysed the effect of glucose concentrations on K^+ and Cl^- by both direct and indirect ISE. In case of K^+ estimation no significant difference was observed between direct and indirect ISE methods, probably due to presence of valinomycin in the ion selective membrane of K^+ electrodes which is highly specific for the ion and thus results are quite transferable between various methods in case of K^+ [12]. Furthermore, increasing glucose concentrations had no significant effect on K^+ levels by either of the methods in serum; this could probably be due to low concentration of the analyte in plasma. So, any change in concentration will also be numerically small enough to produce significant difference. This is further corroborated by the fact that in case of urine sample where the concentrations are almost 10 times higher K^+ shows trend similar to Na^+. Till date not much is known about K^+-glucose interactions and needs further studies in this subject.

The Cl^- levels showed significantly lower values in the direct ISE measurements compared to indirect ISE, an effect similar to Na^+. Most currently employed Cl^- electrodes are not very specific for Cl^- and uses the Nikolsky–Eisenman equation for estimation [13, 14]. This could be the possible reason for getting difference of 11 mmol/L between direct and indirect methods as there could be difference in the selectivity of membrane used in two methods. Decrease in Cl^- levels with increasing glucose concentration in indirect ISE needs further evaluation.

Conclusion

Glucose interferes with the estimation of serum Na^+ and Cl^- by indirect ISE at glucose concentration of 2486 mg/dL. Glucose also interferes with urine Na^+, Cl^- and K^+ at a concentration of 2104 mg/dL. Our findings suggest that in case of very high glucose concentrations found in blood or urine the results of Na^+ estimation by Indirect ISE, the negative bias of Na^+ should be kept in mind.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interests.

Ethical Standards There was no involvement of any direct human or animal subjects in any experiments in the study. All procedures performed in the study involving samples from human origin were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study has also been approved by the Ethical Committee of PGIMER, Chandigarh no NK/2341/Study/4727.

References

1. Tietz NW, Pruden EL, Siggaard-Andersen O. Electrolytes. In: Burtis CA, Ashwood ER, editors. Tietz fundamental of clinical chemistry. 4th ed. Philadelphia: WB Saunders; 2006. p. 95–101.
2. Albert V, Subramanian A, Rangarajan K, Pandey RM. Agreement of two different laboratory methods used to measure electrolytes. J Lab Phys. 2011;3:104–9.
3. Nguyen MK, Ornekian V, Butch AW, Kurtz I. A new method for determining plasma water content: application in pseudohyponatremia. Am J Physiol Renal Physiol. 2007;292:F1652–6.
4. Dimeski G, Mollee P, Carter A. Effects of hyperlipidemia on plasma sodium, potassium, and chloride measurements by an indirect ion-selective electrode measuring system. Clin Chem. 2006;52:155–6.
5. Al-Musheifri A, Jones GR. Glucose interference in direct ion-sensitive electrode sodium measurements. Ann Clin Biochem. 2008;45:530–2.
6. Levy GB. Determination of sodium with ion-selective electrodes. Clin Chem. 1981;27:1435–8.
7. Dimeski G, Badrick T, John AS. Ion selective electrodes (ISEs) and interferences-a review. Clin Chim Acta. 2010;411:309–17.
8. Dimeski G, Barnett RJ. Effects of total plasma protein concentration on plasma sodium, potassium and chloride measurements by an indirect ion selective electrode measuring system. Crit Care Resusc. 2005;7:12–5.
9. Twomey PJ, Cordle J, Pledger DR, Miao Y. An unusual case of hyponatraemia in diabetic ketoacidosis. J Clin Pathol. 2005;58:1219–20.
10. Sbrignadello Stefano, Tura Andrea, Ravazzani Paolo. Electroimpedance spectroscopy for the measurement of the dielectric properties of Na^+Cl^- solutions at different glucose concentrations. J Spectros. 2013. doi:10.1155/2013/571372.
11. Mayes HB, Tian J, Nolte MW, Shanks BH, Beckham GT, Gnanakaran S, et al. Sodium ion interactions with aqueous glucose: insights from quantum mechanics, molecular dynamics, and experiment. J Phys Chem B. 2014;118:1990–2000.
12. Frant MS, Ross JW Jr. Potassium ion specific electrode with high selectivity for potassium over sodium. Science. 1970;167:987–8.
13. Rumenjak V, Milardovic S, Kruhak I, Grabaric BS. The study of some possible measurement errors in clinical blood electrolyte potentiometric (ISE) analysers. Clin Chim Acta. 2003;335:75–81.
14. Wang T, Diamandis EP, Lane A, Baines AD. Variable selectivity of the Hitachi chemistry analyzer chloride ion-selective electrode toward interfering ions. Clin Biochem. 1994;27:14–37.