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Original Research Article

A Comparative Evaluation of Sodium and Potassium Measurements by Flame Photometer and by Direct ISE Methods

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ABSTRACT

Introduction: Electrolyte measurement is very crucial especially for critical patients, but there is lot of apprehensions regarding the reporting of electrolyte owing to different methods used for electrolyte estimation. The study tries to draw an agreement in the sodium and potassium measurement by conventional flame photometer (NFP 460) and much advanced direct ISE (Vitros 350).
Methods: 131 samples were analysed for sodium and potassium by flame photometer (NFP 460) and Vitros 350 dry chemistry analyser. Samples with normal levels of total protein, albumin, globulin, total cholesterol and triglycerides were considered for the study to avoid the interference of high protein and hyperlipidemia proved in various studies.
Results: The study has drawn a considerable agreement in the measurement of sodium and potassium by the flame photometer and the direct ISE with the mean difference ± SD between the two methods for sodium and potassium being 2.47 ± 1.83 and 0.31 ± 0.52 respectively. The study further shows a 95% limit of agreement between the two methods by Bland and Altmant method.
Conclusion: There is an agreement in measurement of sodium and potassium by the two methods and both the methods can be used with equal merits.
Keywords: flame photometer, direct ISE, sodium, potassium

INTRODUCTION

Electrolytes are very crucial parameter in critical care. So, the measurement of the electrolytes accurately is very important for clinical decision making. [1] There has been a considerable change in the methods for estimation of electrolyte in biological samples. Flame photometer, atomic absorption spectrophotometer and direct and indirect ISE are the available methods for estimation of body electrolytes. Barnes, Richardson, Berry, and Hood devised the flame photometer to measure low concentrations of sodium and potassium in solution. [2] Atomic absorption spectrometry, its underlying principles and its analytical techniques were established by Robert Wilhelm Bunsen and Gustav Robert Kirchhoff and it is very often used for electrolyte estimation in biological fluids. [3] These methods are however cumbersome and require manual operations besides having a low throughput. This led to wide research for identifying a technique for electrolyte estimation which will be less cumbersome and deliver accurate and instantaneous results. One such development is the use of Ion Selective Electrode or ISE for estimation of body electrolytes. [4] ISE measures electrolytes by two different technologies. One of them is the direct ISE (direct potentiometry) technology which measures electrolytes in undiluted samples e.g. serum or the plasma component of whole blood. The other type of ISE is the indirect ISE where the sample (e.g. plasma, serum or other fluids) is first diluted with diluent of a certain ionic strength before the concentrations of the electrolytes are measured. [5] There are claims that direct ISE is superior to other methods of electrolyte estimation. [6] There are sufficient studies which compare electrolyte estimation between flame photometer and indirect ISE or between direct and indirect ISE, but there is a paucity of comparison between flame photometer and direct potentiometry methods for electrolyte estimation in Indian settings. Various studies has shown that the presence of hyperlipidemia or hyperproteinemia falsely depress sodium measurement in sample measured either by flame photometer or by indirect ISE. [7, 8, 9] So, in an effort to avoid the already established confounding factors only samples having a normal protein and lipid profile were considered for the study. Thus this study was conducted to do a comparative analysis of the results for estimation of sodium and potassium by the conventional flame photometer method and by the much acclaimed direct ISE in patients with a normal serum protein and serum lipid profile.

The measurement of sodium and potassium was done by flame photometer model no. NFP 460 manufactured by RAC exporters and manufacturer, India. In the flame photometer the solution to be analyzed is discharged through an atomizer in a fine mist into a chamber, where it is drawn into a flame. By an optical system the light produced by the combustion of the elements in the vaporized solution is conducted through appropriate filters to impinge upon a photoelectric cell which activates a galvanometer. Under proper operative conditions the concentration of sodium or potassium in the solution can be estimated from the reading of the galvanometer. The instrument is equipped with an amplifier which permits the analysis of solutions with greatly varying concentrations of sodium and potassium.

For estimation of sodium and potassium by direct ISE Vitros 350 manufactured by Ortho Clinical diagnostic of Jhonson and Jhonson, USA was used. The Vitros 350 dry chemistry analyser estimates sodium and potassium by the principle of direct potentiometry where an individual slide with electrodes measures the potential difference between the electrolytes in the sample and the reference fluid without diluting the sample. The instrument measures the electrolyte concentration in the undiluted serum or the plasma component of whole blood, and so the interference due to larger molecules like proteins or lipids is avoided.

MATERIALS AND METHODS

The present study was conducted in a group of adult individuals of either sex in the age group of 20 and 40 years, and taken randomly from different socio-economic status of north eastern region of India. The study was conducted in the department of Biochemistry in collaboration with the Out Patient Department of Medicine of Gauhati Medical College and Hospital, Guwahati, for a period of three months. Patients and attendants attending the Out patients department of Medicine, Gauhati Medical College were considered for this study.

Clearance from the ethical committee and a written consent was taken from all the participants in the study. Samples were drawn following standard venipuncture techniques and collected in sterile plain vials. A total of 131 samples were analysed simultaneously in the two systems, flame photometer (NFP 460) and Vitros 350 dry chemistry analyser and 18 samples were rejected due to hemolysis, high serum protein or high serum lipid profile.

Samples were allowed to clot and separated immediately after centrifugation. The samples were then divided into two parts and were analysed for Na and K within one hour of collection, in the two analysers, flame photometer (NFP 460) and Vitros 350 system. The samples were analysed by two different personnel to prevent any bias.

Besides sodium and potassium, total protein, albumin, globulin, total cholesterol and triglycerides were measured in all the samples in Vitros 350 dry chemistry system. Only those samples with normal levels of total protein, albumin, globulin, total cholesterol and triglycerides were considered for the study.

Estimation of sodium and potassium by flame photometer

In flame photometer, the principle of Flame Emission Photometry is used for sodium and potassium estimation.

A-Reagent preparation and storage

1. NaCl stock standard (NaCl SS) of 1 M concentration was prepared and stored at room temperature.
2. KCl stock standard (KCl SS) of 100 mM concentration was prepared and stored at room temperature.
3. Li_2CO_3 stock standard (Li_2CO_3 SS) of 1 M concentration in 2 M NaOH solution was prepared and stored at room temperature.
4. Working Li_2CO_3 solution (Li_2CO_3 WS) – 15 ml of Li_2CO_3 SS was taken in a 1 L volumetric flask and volume was made up to the mark with deionized water.
5. Working NaCl solution (NaCl WS) and working KCl solution (KCl WS) – 140 ml of NaCl SS and 5 ml of KCl SS were taken in 1 L volumetric flask and then the volume was made up to the mark by adding deionized water. This way 140 mmol/L Na^+ and 5 mmol/L K^+ standard was made. Similarly other higher and lower concentration standards were made for calibration of the flame photometer.

B-Sample preparation and Dilution

1. Both the serum sample and the Na^+ and K^+ WS were diluted with Li_2CO_3 WS in 1:100 ratio.
2. For sample dilution, 100 µl of serum sample was taken in a test tube (thoroughly rinsed with deionized water and dried) and made the volume up to 10 ml with Li_2CO_3 WS.
3. For Na^+/K^+ WS dilution , 1.5 ml of Na^+/K^+ WS was taken in a beaker (thoroughly rinsed with deionized water and dried) and made the volume up to 150 ml Li_2CO_3 WS.

   Then the sample are analysed by the flame photometer.

Estimation of sodium and potassium by Vitros 350 auto-analyser

The other half of the sample is directly allowed to be analysed in the Vitros 350 dry chemistry system, without any further processing.

All the data is documented on a excel sheet to show the difference in the values of serum Na^+ and K^+ measured in the flame photometer and Vitros 350 system respectively. Bland and Altman technique is used to describe the agreement between two methods of electrolyte measurement on the same sample. The 95% limits of agreement are estimated by mean difference ± 2.0

standard deviation of the differences in the measurement by the two methods. The result of sodium and potassium measurements by the two instruments is shown by mean ± SD.

RESULTS

In total 131 samples were considered for the study. Table 1 shows the mean of serum sodium and potassium measured in flame photometer (NFP 460) and Vitros 350, respectively.

Table no. 1. Mean of serum sodium and potassium measured in flame photometer and Vitros.

Parameter	Mean in flame photometer	Mean in Vitros 350
Serum sodium (mmol/L)	141.80 ± 4.72	144.26 ± 5.55
Serum potassium (mmol/L)	4.23 ± 0.49	4.54 ± 0.39

Figure 1 and figure 2 Shows the measurement of serum sodium and serum potassium respectively, using flame photometer and plotted against that measured by the Vitros 350. The graphs also show the line of equality. This makes it easier to assess visually how well the methods agree.

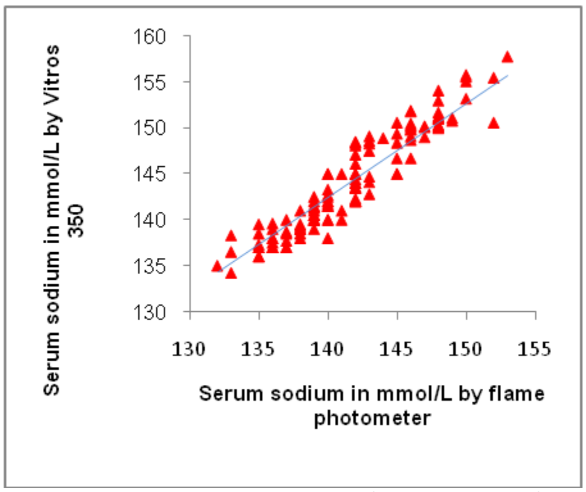

Figure 1. Graph showing the comparison of serum sodium by flame photometer and Vitros 350 systems.

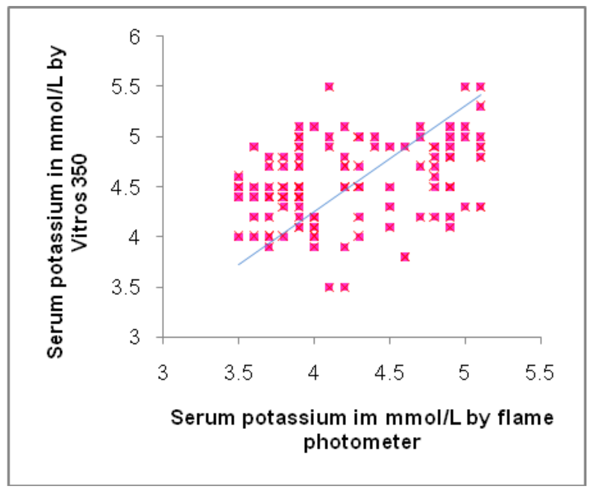

Figure 2. Graph showing the comparison of serum potassium by flame photometer and Vitros 350 systems.

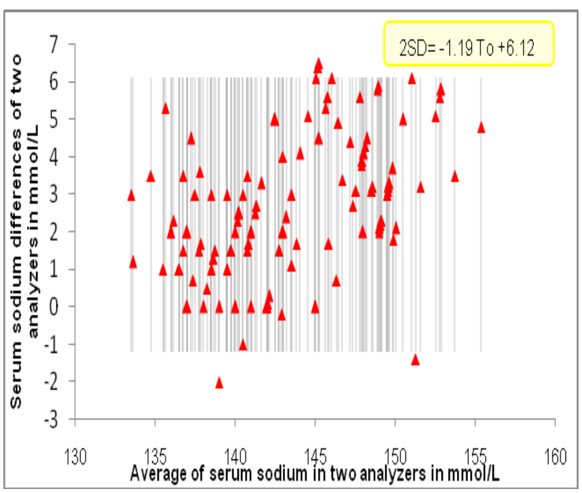

Figure 3.Graph showing the comparison of average of serum sodium by two analyzers against their differences.

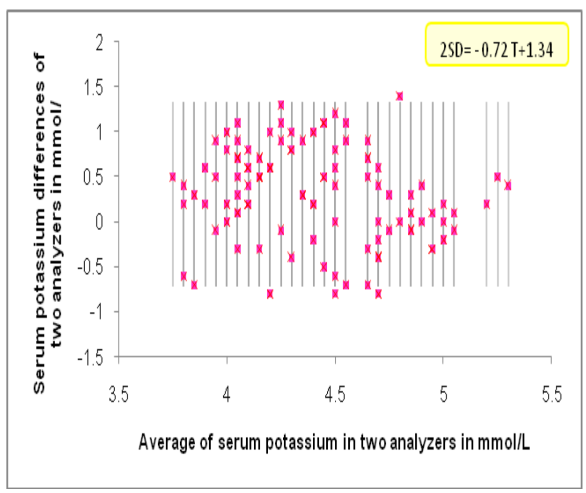

Figure 4. Graph showing the comparison of average of serum potassium by two analyzers against their differences.

To verify the agreement of flame photometer with direct ISE the difference in the measurement of serum sodium and potassium by the two methods were calculated. The mean difference ± SD for sodium and potassium are 2.47 ± 1.83 and 0.31 ± 0.52 respectively. These values are then used for Bland and Altmant's methods for determining the agreement. Figure 3 and figure 4 features a better way of displaying the data by plotting the difference between the two analysers measurements for each sample against their mean. The plot will also show clearly any extreme or outlying observations. 95% limits of agreement (2SD) to this plot are added to provide a good summary picture.

DISCUSSION

The measurement of electrolytes is very vital for clinical decision making in medical science. The importance of electrolyte is more so in critically ill patients. [1] So the present day medical practice demands a precise and urgent reporting of serum electrolytes in almost every case. There has also been a revolutionary achievement in the ways of managing dyselectrolytemia in medical practice which has also contributed to the importance of urgent electrolyte reporting. Point of care (POCT) testing of electrolyte is widely practiced in many set ups, more so in intensive care units. But India is a country where there is a great variance in the quality of medical care, from world class facility in cities to a lack of basic medical care in villages. As far as electrolyte estimation is concerned when POCT is the rule in some highly sophisticated center of India simultaneously flame photometer is also used in some ill equipped center. We made an attempt to bring a comparative agreement between electrolyte reporting by a conventional instrument like flame photometer and most advanced direct ISE method. It is also the first study of its kind in the north eastern part of India.

We have used the Bland and Altman agreement analysis for the data obtained. [10] The 95% limits of agreement, estimated by mean difference ± 2 standard deviation of the differences, provide an interval within which 95% of differences between measurements by the two instruments are found to lie. However there are 5% chances that these values may lay outside the limit of agreement. We can then say that nearly all pairs of measurements by the two instruments will be closer together than the extreme values, which we call 95% limits of agreement. The calculation of the 95% limits of agreement is based on the assumption that the differences are normally distributed. Such differences are, in fact, quite likely to follow a normal distribution. The study therefore proves that there is appreciable agreement in the measurement of electrolyte by flame photometer and direct ISE in patient with a normal serum protein and normal lipid profile.

The basic difference of flame photometer and direct ISE is the electrolyte measurement in diluted and undiluted sample respectively. [5] So it is always expected that there will be some difference in the measured value of electrolyte by the two instruments. The CLSI (Clinical Laboratory and Standards Institute, formerly known as the NCCLS) recommends that ion activity results obtained by the direct ISE methodology be adjusted to resemble those obtained by procedures that measure plasma concentration. [11] Most instruments using direct ISE have built-in conversion algorithms that give results in concentration terms that are comparable to the reference method (flame photometry) for specimens with normal plasma water.

The effect of a high concentration of protein and lipid on sodium measurement by indirect ISE or flame photometry has been reported previously, with both high levels of protein and lipid concentrations resulting in pseudohyponatraemia.[5,6,9] Direct ISE technology eliminates this artifact. [6] The findings of the present study therefore is in agreement with previous findings and proves that there is no significant difference in the electrolyte measurement by flame photometer and direct ISE in subjects with a normal protein and lipid profile. Thus flame photometer can be a suitable instrument for reporting electrolyte values in small setups of India, a suggestion which was given by H G Worth more than a decade ago. [12]

CONCLUSION

The study reveals that there is a good degree of agreement when electrolyte is measured using conventional flame photometer and direct ISE. So this study is expected to build trust in electrolyte reporting done by flame photometer in most remote centers of India.

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