A study comparing values of serum sodium estimated by colorimetric kit method with those obtained by direct and indirect ion selective electrode methods

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Abstract

Introduction: Electrolyte abnormalities are known to cause significant morbidity and mortality, when it is not detected. Dyselectrolytemias are usually treatable. Hyponatremia is a common manifestation encountered in the elderly. The rural health care facilities may not have well equipped laboratories. Therefore, this study intended to look for the possibility of establishing a screening technique that can help detect hyponatremia in such centres.

Materials and Methods: Serum sodium levels were estimated in 120 samples using direct, indirect ISE and colorimeter. Statistical analysis was done using SPSS version 17, NCSS 11 and MINITAB 18 software. A p value< 0.05 was considered statistically significant.

Results: A simple linear regression analysis was done and a regression equation was derived for sodium levels while comparing between the instruments. The Bland Altman analysis gave an agreement limit of 95% between the instruments which is well within the CLIA suggested target value of ±4 mmol/L in the hyponatremic and normonatremic range.

Conclusion: The derived regression equation calculates a predicted value for direct and Indirect ISE using the values obtained on the colorimeter making it comparable on all the three instruments in the hyponatremic and normonatremic ranges. The colorimetric method can be used as a cost effective screening technique to identify the hyponatremic elderly for whom a tertiary hospital may not be accessible, thus facilitating early intervention.

Keywords: Serum sodium, Direct ISE, Indirect ISE, Colorimetry, Hyponatremia.

Introduction

Sodium is the major extracellular cation located in the extracellular fluid. It maintains the osmotic pressure and acid-base equilibrium and also maintains the tissue hydration.[1]

Serum sodium is a crucial parameter in critical care management. Hence, the accurate measurement of levels of sodium is very important in taking clinical decisions for treating patients. Over the years there has been a lot of progress in terms of use of newer methods for accurate and faster estimation of sodium in blood and serum. The earliest method for estimating serum sodium was based on the formation of a salt with Zinc uranyl acetate, followed by gravimetric, titrimetric or colorimetric quantitation as described by E.C Butterworth.[1,2,4] Later Barnes, Richardson, Berry & Hood devised the flame photometer to measure low concentrations of sodium in solution.[3] Atomic absorption spectrometry was established by R W Bunsen, Gustav & R Kirchhoff for estimation of electrolytes in various biological fluids. However, most of the above methods have a long turnaround time, less sample throughput and are cumbersome to run.[3] With the advent of newer methods, the Ion Selective Electrode (ISE) came into being and this can specifically measure sodium in a short span of time.[5] This is the current standard reference method.

Several studies have been conducted so far comparing the various methods for estimation of electrolytes[3,6-9] A study[6] showed that analytical performance of colorimetric method is acceptable for sodium and potassium while the analytical performance of flame photometry was not acceptable, keeping the ISE as the reference method. The studies[3,7] compare the values of sodium measured by flame photometer against the direct & indirect ISE. Both the studies indicate that there is a good agreement between the two methods and both can be used interchangeably. The studies[8,9] compare the electrolyte values measured on an auto analyser and the arterial blood gas instrument. Both these studies observe that the clinicians ought to be cautious while using the values interchangeably.

The values of sodium measured on an ISE though accurate, is expensive. Hyponatremia is a treatable cause and must be detected in the early stages for better prognosis. This study aims to compare serum sodium values estimated by colorimetric kit method with the values obtained by direct and indirect ISE methods. Colorimetric estimation is cost effective and this simple instrument can be placed in any Primary health care centre (PHC). This study was undertaken to evolve a logistically feasible, inexpensive methodology/ instrument for electrolyte estimation.

Materials and Methods

Study Design: Prospective Study

Time and Place of Study: The study was conducted for a period of 09 months from Aug 2016 to April 2017

in the Clinical Chemistry Section of the Department of Laboratory Medicine, Ramaiah Medical College Hospital, Bengaluru.

Inclusion & Exclusion Criteria: 120 serum samples of patients aged between 18–80 years of either gender, sent to the lab with a request for electrolyte estimation was included in the study. Samples which were haemolysed, turbid, lipemic or hyperbilrubinemic were excluded.

Ethical Approval & Informed Consent: The Ethical Clearance was obtained from the Ethics Committee of the Medical College and a written informed consent was taken from every patient or his relatives who came with the request for electrolytes. (Enclosed)

Methods: The samples collected with a request for electrolytes were analysed using indirect ISE on Roche Cobas c501 chemistry analyser (Roche Diagnostics GmbH, Mannheim, Germany) or using direct ISE on Roche AVL 9180 (9180) electrolyte analyser (Roche Diagnostics). Sodium was then measured within 2hrs in the same serum sample using sodium electrolyte colorimetric test kit obtained from Excel Diagnostics Pvt. Ltd. (Hyderabad, India).

Principle: Both direct and indirect ISE are based on Potentiometry. There is a change in the electromotive force in a circuit between a measurement electrode and the reference electrode, as the selected ion interacts with the membrane of the ISE (10). Indirect ISE measures sodium by diluting the sample. The samples are diluted in ratios of 1:20 to 1:34 (10, 11) depending on the analytical system (9.7µl of sample + 291 µl of ISE Diluent in Roche Cobas c501). In the direct ISE method, the sample is presented to the electrodes without dilution.[10,11] Colorimetrically, serum sodium was estimated using the method of Trinder.[12] The Trinder's method[12] used in the present study makes use of a two-step process. The first step involves the precipitation of sodium and proteins using a sodium precipitating reagent which contains magnesium uranyl acetate (20µl of serum + 1000 µl of precipitating reagent). On mixing with the precipitating reagent, the mixture is centrifuged for 1 full minute at 3000rpm. In the second step, 50 µl of the supernatant which contains excess uranyl salts is reacted with 200 µl colour reagent containing potassium ferricyanide resulting in formation of dark brown coloured ferrous uranyl acetate which is read at 540 nm after 5 minutes. The intensity of the colour produced is inversely proportional to the amount of sodium present in the serum sample. Blank is also run along with the test sample making use of distilled water in place of serum. A standard was run on the colorimeter every day prior to running the patient's sample to look for precision. Precipitation of sodium is an important step.

Statistical Analysis and Results

120 serum samples were collected by simple random sampling. Sodium (Na^+) was estimated on two different instruments either on Roche Cobas (c501) or Roche AVL 9180 (9180) by Indirect ISE and Direct ISE methods respectively. Of the 120 samples, 100 were compared between C501 and colorimetry and 35 samples was compared between 9180 and colorimetry. Data was entered on MS Excel Sheet and the statistical analysis was done using SPSS version 17, NCSS 11 & MINITAB 18 data analysis statistical software. P value <0.05 was considered as statistically significant.

The Na^+ values obtained from C501 & 9180 were further classified into 3 groups (Table 1) based on the reference ranges.[15] The values of sodium were presented using descriptive statistics such as mean, S.D and CV (Table 2). Correlation was computed for Na^+ values, using Pearson's correlation coefficient (Table 3) and Scatter plots were drawn to depict the same (Fig. 1a & 1b). A simple linear regression[14] was done for sodium values between the two instruments (C501vs Colorimetry & 9180 vs Colorimetry). The linear regression equation was derived for Na^+ values obtained on a colorimeter to predict the values on c501 and 9180 and see whether they form a model of good fit (Table 4 & Fig. 2 & 3). The number of samples comparable between C501 and colorimeter in the hyponatremia, normonatremia, and hypernatremic ranges were 48, 41 and 4 respectively while that between 9180 and colorimeter in the hyponatremia, normonatremia, and hypernatremic ranges were 29, 06 and zero respectively.

The values for sodium were compared only in the hyponatremic and normonatremic ranges (Table 5) owing to the fact that samples for this study was collected by simple random sampling and the number of samples obtained in the hypernatremia range was insufficient to give any statistically significant data.

Table 1: Reference ranges for serum sodium[15]

S. No.	Analyte	Reference Ranges (mmol/L)	Groups
1.	Sodium (Na^+)	136 – 145	Normonatremia
2.	Sodium (Na^+)	<135	Hyponatremia
3.	Sodium (Na^+)	>145	Hypernatremia

Table 2: Mean, S.D and CV% for sodium levels between C501, 9180 and colorimetry

	N	Mean	Std. Deviation	CV%
Na^+ c501	100	135.30	10.41	7.69
Na^+ Colorimetry	100	146.22	18.16	12.42
Na^+ 9180	35	131.31	9.91	7.55
Na^+ Colorimetry	35	140.53	16.57	11.79

Table 3: Pearson's correlation between Na^+ c501 vs colorimetry & Na^+ 9180 vs colorimetry

	N	Na^+ on Colorimetry	P value
Na^+ on c501	100	0.596	<0.001
Na^+ on 9180	35	0.547	<0.001

Table 4: Simple linear regression between instruments and ANOVA for unstandardized predicted values

	R	R^2	Prediction Equation	ANOVA for Unstandardized Predicted Values
Na^+C501 vs Colorimeter	0.596 P<0.001	35.58% P<0.001	Na^+ on C501 = 85.13 + 0.3426 Na^+ by colorimetry	F=169.43, P<0.001
Na^+ 9180 vs Colorimeter	0.547 P<0.001	29.95% P<0.001	Na^+ on 9180 = 85.3 + 0.3274 Na^+ by colorimetry	F=42.85, P<0.001

Table 5: Na+ levels between the instruments in hyponatremia & normonatremia

	Na^+ C501 vs Na^+ Colorimetry	Na^+ 9180 vs Na^+ Colorimetry	Na^+ C501 vs Na^+ Colorimetry	Na^+ 9180 vs Na^+ Colorimetry
Na^+ Levels	**<135 mmol/L**	**<135 mmol/L**	**135-145 mmol/L**	**135-145 mmol/L**
Total % of samples	42.00%	57.14%	48.00%	37.14%
% of Comparable samples	69.05%	60.00%	58.33%	92.31%
R (P<0.001)	0.751	0.88	0.511	0.39
R^2 (P<0.001)	56.34%	78%	26.13%	15.62%
Prediction Equation	Na^+ on C501 = 55.04+0.54 Na^+ by colorimetry	Na^+ on 9180 = 19.67+0.83 Na^+ by colorimetry	Na^+ on C501 = 112.1+0.19 Na^+ by colorimetry	Na^+ on 9180 = 113.5+0.17 Na^+ by colorimetry
Bland Altman Analysis (Mean Difference)	-3.01	-1.66	-3.17	-2.59
Limits of Agreement [defined as Difference ± 1.96 * standard deviation (SD)] by Bland Altman analysis	-15.06 to 9.03 mmol/L	-11.92 to 8.59 mmol/L	-15.65 to 9.30 mmol/L	-10.64 to 5.47 mmol/L

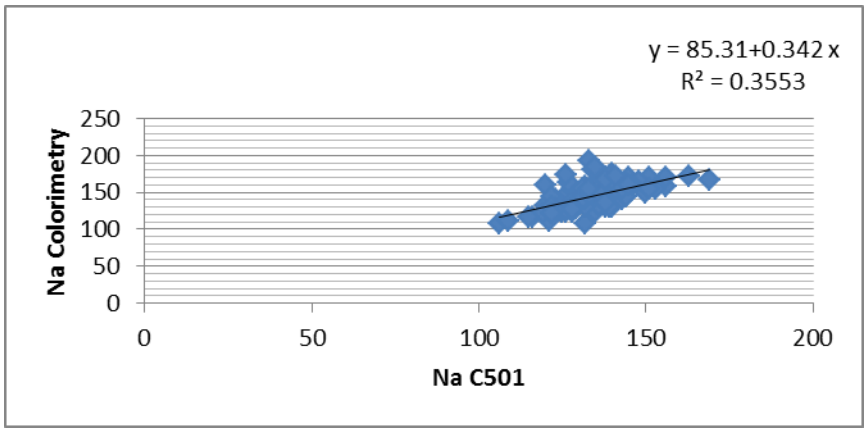

Fig. 1a: Scatter plot for Na^+ c501 vs colorimetry

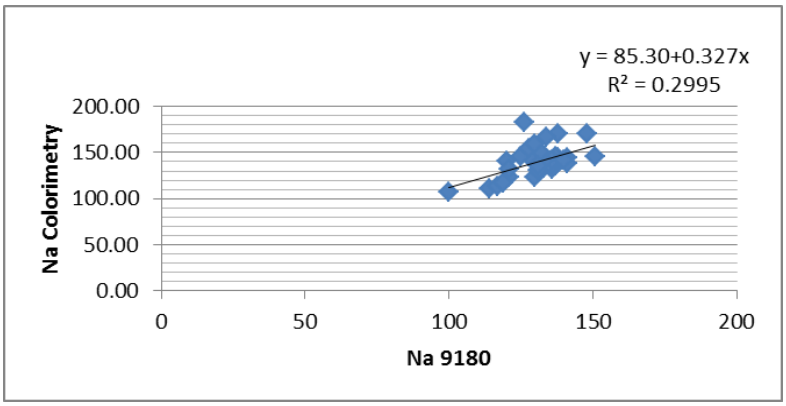

Fig. 1b: Scatter plot for Na^+ 9180 vs colorimetry

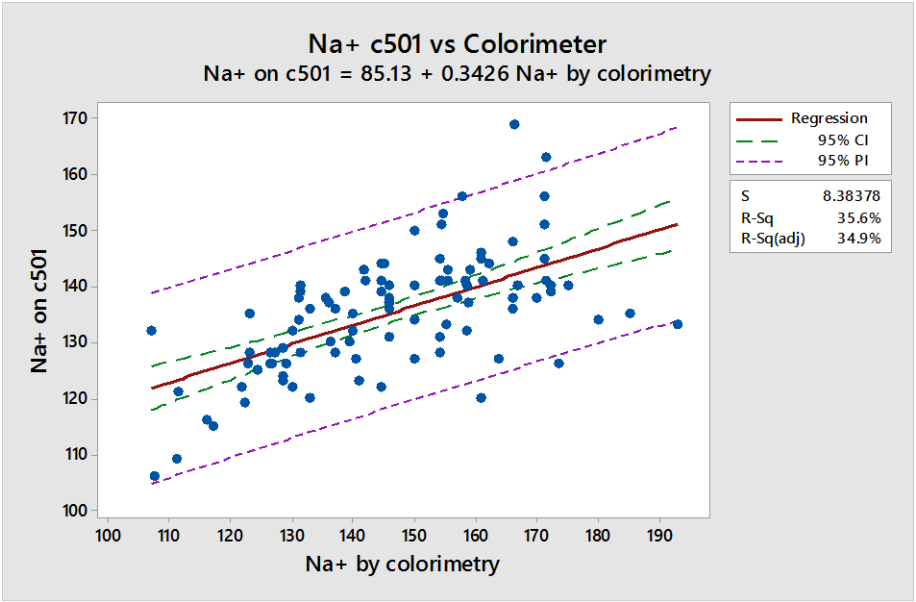

Fig. 2: Simple linear regression for Na^+ values between c501 and colorimeter

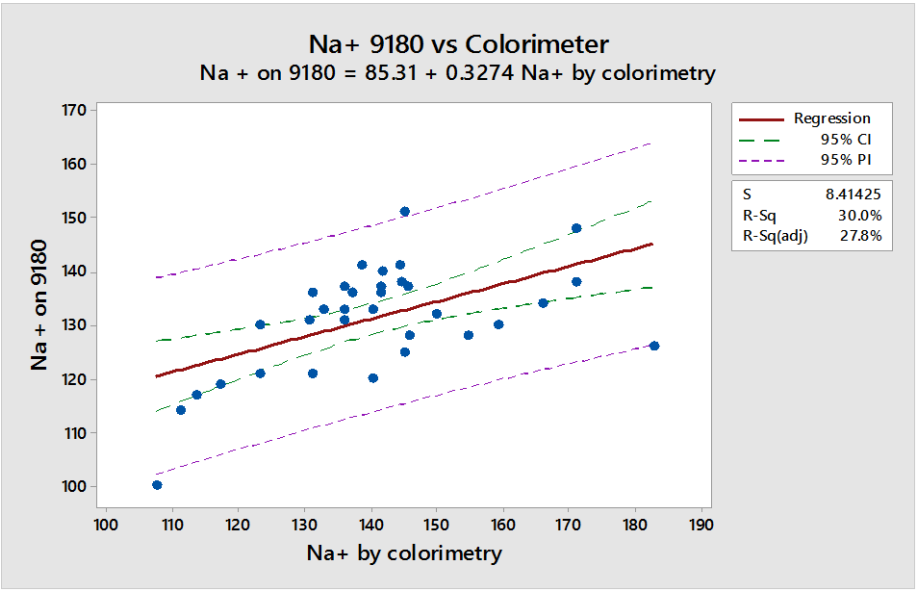

Fig 3: Simple linear regression for Na^+ values between 9180 and colorimeter

Discussion

Serum sodium is the biochemical analyte which is commonly measured in patients who are either admitted in the critical care unit or those attending the outpatient department for a routine health check. Most tertiary care hospitals have diagnostic laboratories that use ISE[11] for sodium estimation. Dysnatremia, a treatable cause is often missed in the peripheral/rural health centres due to lack of instrumentation. Though ISEs can give the results in a very short span of time, it is expensive and may not be logistically feasible to use them in every primary health centre. In such instances, rural population will have to seek facilities available only in the urban areas to detect hyponatremia. To overcome the above problem, the present study measured serum sodium using a simple cost effective instrument viz. Colorimeter. The same was compared with the values obtained by the use of ISEs.

Sample Size with Proper Justification: A study[7] carried out on "Comparison of Ion selective electrode, flame emission spectrophotometer and colorimetry in the determination of serum electrolyte", has revealed the findings of analysis of various methods. Sodium levels at different concentrations of 100, 135 & 175 mmol/L, when measured using ISE method were 92.50 (1.84), 125.8 (1.14) & 166.50 (2.55) {Mean (SD)} as compared to that measured by colorimetric method, which gave comparable values of 102.8 (0.42), 134.8 (0.42) & 175.70 (0.48). Based on the above findings and considering an α-error of 1%, a sample size of 10 was found to be sufficient for statistical significance and to achieve a power of 99%. However, it was proposed to include a larger sample size taking into consideration, the manual errors associated with precipitation of sodium in the colorimetric method and to achieve a desirable precision between the instruments.

Table 3 shows that there is a moderately positive linear correlation between C501 and colorimeter & 9180 and colorimeter for sodium values. A simple linear regression[14] was applied between the methods/instruments (Indirect ISE/c501 vs colorimetry & direct ISE/9180 vs colorimetry) to see if the values of sodium were comparable. The ANOVA for Unstandardized predicted values (Table 4) between c501 and colorimeter and 9180 and colorimeter, for Na^+ values shows an F value = 169.429 & 42.85 with $P < 0.001$. This indicates that the sodium values are comparable between the instruments and the regression model is a good fit (Fig. 2 & 3) for the data obtained. The regression equations calculated using the R^2 value for sodium by colorimetry to predict the value on c501 = 85.13 + 0.3426 (Na^+ by Colorimetry) & for that on 9180 is 85.3 + 0.3274 (Na^+ by Colorimetry). In other words, for every 1 mmol/L increase in Na^+ by colorimetry, the value of Na^+ increases by 0.3426 mmol/L on c501 & 0.3274 mmol/L on 9180 respectively.

The Bland Altman analysis done (Table 5) indicates that the mean difference of Na^+ values was beyond the CLIA suggested target value ±4mmol/L.[15] Hence a further analysis was done in the hyponatremic and normonatremic ranges. Table 6 shows that the Pearson's correlation between the instruments is strongly positive in the hyponatremic range and moderately positive in the normonatremic range. The prediction of Na^+ values on c501 and 9180 using the regression equation is 56.34% & 78% in hyponatremic range and 26.13% & 15.62% in normonatremic range indicating better prediction in hyponatremic ranges. The Bland Altman Analysis of the above values shows the mean difference for Na^+ levels to be well within the CLIA suggested ±4 mmol/L (Table 5).

Hypo & Hypernatremia are often asymptomatic in the mild stages. They mimic a central nervous system disorder presenting with symptoms of irritability, nausea, weakness, disorientation, seizures and so on.[16] The symptoms of hyponatremia vary depending on the severity and the rate of sodium decline.[16] A high level of suspicion is necessary to pick up either hyponatremia or hypernatremia. Both are associated with increased risk of mortality and morbidity.[16] If a cost effective instrument like a colorimeter is used in a rural health care setup, it can help detect dysnatremia in the mild stage facilitating early intervention, thus improving the quality of life.

Limitations of our Study

1. Serum sodium levels are known to be affected by glucose, protein and lipid concentrations. Lack of estimation of the above components is a major limitation in our study.
2. The same sample was not analyzed for sodium on both c501 and 9180 (except for about 15 samples) as it is an established fact that both direct and indirect ISE give comparable results.
3. Estimation on a larger sample size would have evaluated the sensitivity and specificity of the Sodium colorimetric kit. Further, the study lacked a statistically significant number of hypernatremic samples owing to random sampling.

Merits of the Study: This is a pilot study that has made an effort to high light the usefulness of a simple inexpensive instrument in detecting hyponatremia in rural health care centres, thus facilitating timely medical aid decreasing the incidence of morbidity and mortality that is associated with it otherwise.

Conclusion

1. The values of sodium estimated by both the instruments (Direct & Indirect ISE) are comparable with that obtained on a colorimeter in hyponatremic and normonatremic ranges.

2. The regression equations have been derived to predict Na^+ values on C501 and 9180 when the samples are processed using a colorimeter.
3. Screening for a treatable condition like hyponatremia, especially in the geriatric age groups, using a cost effective colorimeter, in the rural areas will provide an opportunity to avail early interventional therapy.

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