Original article
To Compare Creatinine Estimation by Jaffe and Enzymatic Method

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ABSTRACT

INTRODUCTION: Creatinine in urine and serum are used in the assessment of renal function. It is commonly estimated by Jaffe's and enzymatic method. In many institutions, serum creatinine is estimated by (POCT) Point of care testing device (Enzymatic method) and follow up of patients with creatinine results by other method, analyzed in the Biochemistry laboratory. If the results of POCT does not correlate with the Jaffe method, it leads to differences in serum creatinine values and wrong treatment decisions during follow up of patients. Hence, this study was done to know the difference between two methods in a tertiary care hospital. AIM AND OBJECTIVE: To estimate creatinine by Jaffe's and Enzymatic method and to compare the serum creatinine values between the two methods. MATERIALS AND METHODS: It is an observational cross-sectional study, for a period of 17 months from Nov 2018 to March 2020. 75 samples were analyzed for serum Creatinine by Jaffe's method in Biochemistry laboratory and by enzymatic method in emergency department in POCT device. RESULTS: Mean difference between Jaffe's and Enzymatic method were -0.063 mg/dL, 0.070 mg/dL, 0.198 mg/dl and 0.0685 in group I, II, III and all the group together. The overall Intra class correlation coefficient including all the three groups (0.995) indicates a very good correlation between the two methods. CONCLUSION: Our study showed a good agreement and good correlation between two methods, which is similar to other studies analyzed on same instrument.

Introduction

Diseases of the kidneys are amongst the most important causes of death and disability in many countries throughout the world.[1,2] Serum creatinine is one of the most commonly measured parameters to assess the renal functions in clinical chemistry laboratories. Currently, there are several methods available for creatinine estimation. Jaffe's method based on alkaline picrate method, with few modifications to remove interference, is most commonly used for creatinine estimation first described by Jaffe in 1886.[3] The other methods are enzymatic methods and Isotope dilution-liquid chromatography-mass spectrometry (IDLCMS) method (gold standard method).[4] Although several methods have been described, the classic Jaffe reaction is most widely used.[5] Expert professional bodies have recommended that all creatinine methods should be traceable to a reference method based on isotope dilution mass spectrometry (IDMS) to harmonize the results among all the laboratories. [6]

Creatinine is also estimated by point of care testing devices, as there is a need for faster test results for treatment. There are several drawbacks with advantages associated with this point of care testing devices. Where whole blood creatinine is estimated on the Radiometer ABL800 FLEX blood gas analyzer, by using Amperometric biosensor (electrode) based on the enzymatic conversion of creatinine to sarcosine with generation of hydrogen peroxide.[7] The enzymatic assay is less prone to non-specific bias than the Jaffe assay, but it is considerably more expensive. Hence, most Laboratories consider creatinine measurement by Jaffe method to minimize the cost of the test.[8]

In several tertiary hospitals, creatinine is estimated by enzymatic method available in (POCT) point of care testing device in emergency setting and treatment decision are considered based on that. In condition where there is doubt regarding results by POCT device, samples are sent for creatinine estimation by Jaffe method in Biochemistry laboratory, for further follow up and treatment. This leads to repeat test, add on cost to the patient and delay in treatment. To avoid this, our study was designed to estimate serum creatinine by Jaffe's method in Biochemistry laboratory and enzymatic method by POCT device and to compare creatinine results between two methods to check the differences.

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MATERIALS AND METHODS:

The study was conducted in tertiary care hospital, Bangalore, after obtaining Institutional ethical review board clearance. 62 samples were required for the study as per the statistical analysis. It was an Observational Cross-Sectional Study for a period of 17 months from November 2018 to March 2020. The patients who have undergone ABG analysis in the Emergency Department and also serum creatinine estimation at the same time in the Biochemistry laboratory are included. The data of all the above patients were collected from the records of various departments of Nephrology, Emergency medicine and Biochemistry Departments. Hemolyzed, wrong labelling, icteric, lipemic samples and the creatinine results from the instruments due to analytical error were excluded. Finally, 75 samples were included in the study.

Serum Creatinine was estimated by Jaffe method on Abbot Architect ci 8200 auto analyzer, in Biochemistry department, in which creatinine reacts with picric acid in alkaline medium and produces orange creatinine picrate color which is measured at 500 nm. Whole blood creatinine was estimated in Arterial blood Gas analyzer (ABL 800 FLEX) by enzymatic method, where creatinine in whole blood is acted upon by creatinine amidohydrolase, finally reducing to hydrogen peroxide and further to oxygen, hydrogen and electrons. Amperometry principle is used to measure the amount of creatinine in the sample. The creatinine results between the two methods were compared to check the agreement between them using Bland-Altman plot. Statistical analysis was done using the Statistical Package for Social Sciences (SPSS) software version 23.

RESULT:

This study was conducted in tertiary care hospital. The creatinine results by both the methods were divided into 3 groups based on clinical decision limits as per the nephrologist opinion, to see the agreement at different levels. The groups are Group I comprise of 25 samples (<2mg/dL), group II comprises 25 samples (2-6mg/dL) and group III comprises 25 samples (> 6mg/dL).

The mean, standard deviation, mean difference and Inter class correlation between whole blood (Radiometer ABL 800 FLEX) and serum creatinine (Abbott Architect ci 8200) is shown in Table 1. Intra class correlation coefficients for the agreement between the two methods including all the groups is 0.995. Method comparison between the enzymatic creatinine method and Jaffe's method by linear regression for all groups together (n=75) showed a coefficient correlation R of 0.9893 (Figure 2d). Figure 1and figure 2 explains the Bland – Altman plot for agreement and scatter plot for correlation between the two methods in each group and all the groups together.

We also analyzed few samples which had flags for creatinine results in ABG analyzer, which are used to treat the patients. The mean difference and ICC is -0.220 mg/dL and 0.894 respectively between the two methods as shown in table 2. Figure: 3a &b. is the bland – Altman plot showing higher mean difference and Regression analysis with correlation coefficient R of 0.7995 indicating lower correlation in comparison with the samples having no flags.

Table 1: Mean, SD and mean difference for the two methods in the four group.

Groups	Methods	Mean mg/dL	SD mg/dL	Mean difference mg/dL (95% CI)	ICC (95% CI)
Group I (n=25) (< 2 mg/dL)	Jaffe kinetic	1.189	0.426	-0.063 (-0.318,0.192)	0.944 (0.879, 0.975)
	Enzymatic	1.252	0.423		
Group II (n=25) (2-6 mg/dL)	Jaffe kinetic	3.436	1.306	0.070 (-0.54, 0.681)	0.971 (0.936, 0.987)
	Enzymatic	3.365	1.302		
Group III (n=25) (> 6 mg/dL)	Jaffe kinetic	10.809	3.633	0.198 (-1.287,1.683)	0.979 (0.953, 0.911)
	Enzymatic	10.611	3.855		
All the Groups (n=75)	Jaffe kinetic	5.144	4.691	0.0685 (-0.881,1.018)	0.995 (0.991,0.997)
	Enzymatic	5.076	4.659		

ICC: <0.2 Poor agreement, 0.21 – 0.4 Fair agreement, 0.41 – 0.6 Moderate agreement, 0.61 – 0.8 Good agreement and 0.81 – 1 Very good agreement,

SD – standard deviation, CI – confidence intervals

Table 2: Mean, SD, mean difference and ICC for the two methods for the Flagged samples.

Numbers	Methods	Mean mg/dL	SD mg/dL	Mean difference (95% CI)	ICC (ρ) (95% CI)
S. Creatinine n= 56 (0.68 -20.35) mg/dL	Kinetic Jaffe	5.710	4.846	-0.220 (-4.530,4.091)	0.894 (0.827,0.937)
	Enzymatic	5.930	4.693		

**SD – standard deviation, ICC – Interclass correlation**

Fig 1: Bland Altman plot between Jaffe's and Enzymatic Method Fig1a (0-2mg/dL), Fig1b (2-6mg/dL), Fig1c (>6mg/dL), Fig1d (All the groups together) SD – Standard Deviation
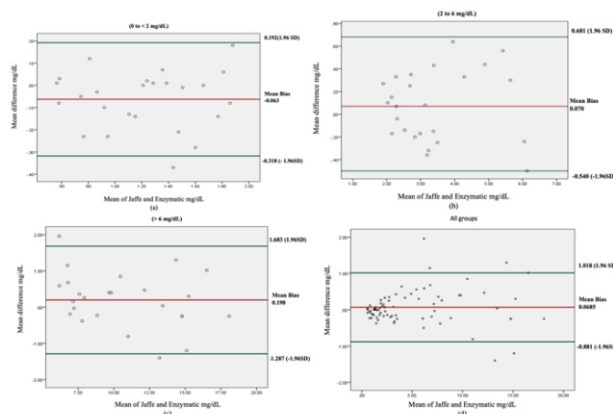
Fig 2: Correlation graph showing the association between two methods Fig2a (0-2mg/dL), Fig2b (2-6mg/dL), Fig2c (>6mg/dL), Fig2d (All the groups together)
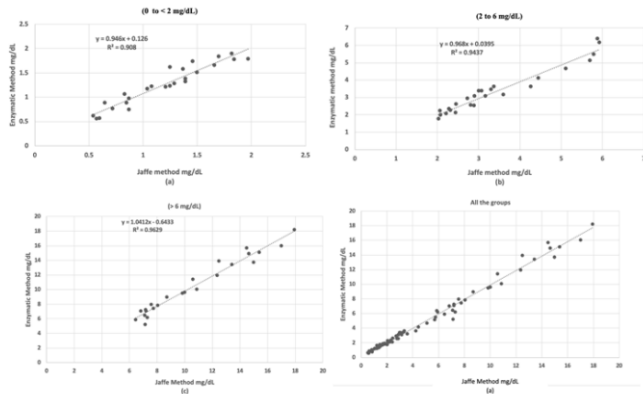
Figure:3a & b Correlation graph and Bland Altman plot showing the strength of the relationship and agreement and between two methods for results showing flags on ABG analyzer.
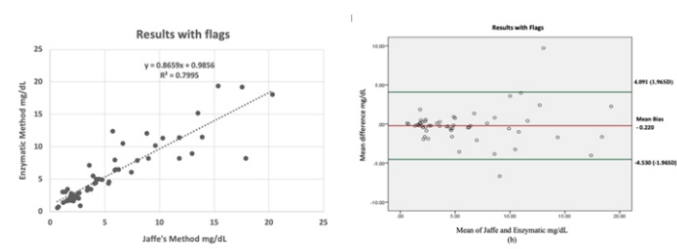
DISCUSSION:

Acute or chronic renal disease is a major health problem all over the world. Estimation of serum/whole blood creatinine levels is required to asses renal function.9 The availability of a rapid creatinine assay by point of care testing device with inaccurate values may allow differential treatment decisions to be made for patients at risk of developing renal failure.10 Clinical studies are needed to demonstrate whether whole blood creatinine assays at point of care testing devices are accurate enough to provide reliable estimates of renal function in their settings.

In our study, the mean difference between Jaffe's and Enzymatic method in all the group together is 0.0685 mg/dL. ICC of 0.995 between the two methods showed very good correlation (Table 1). Vijaya Marakala11 evaluated 167 normal samples (group1) and did not find a significant difference (-0.042) and the ICC also showed a very good correlation (0.995) between two methods.. All the measurements were performed using an Olympus AU 400 analyzer.

Malukar, et al12 showed that enzymatic method has greater linearity (up to 60mg/dL) than the Jaffe's (up to 20mg/dL) Method. This study was done on six groups, each group comprises 66 samples and both the methods were done using ROBONIC Semi auto analyzer. First three groups compared creatinine between two methods and

other three groups were compared for interferences such as glucose, bilirubin and both together. There was very good correlation for serum creatinine (ICC 0.931,0.998,0.986 in group I, II, III respectively) between two methods in all the three groups.

Anne-Sophie et al proved that in comparison of three POCT devices (ABL 800, i-STAT, Stat sensor), ABL 800 creatinine presented excellent agreement with the IDMS-traceable Roche enzymatic assay with a small bias.13 The i-STAT creatinine overestimates14 whereas the Stat Sensor15 underestimates plasma creatinine in comparison to the IDMS-traceable Roche enzymatic assay. ABL 800 FLEX is the instrument used in our setting as POCT device, where the results had good agreement with the Jaffe method. Similar studies were not found which compared creatinine between Jaffe method in fully automated analyzer and enzymatic method by Point of Care Testing device.

In our study in the group III the mean difference is high (0.198 mg/dL) as compared to other two groups (-0.063 mg/dL, 0.070 mg/dL). This may be due to higher range of serum creatinine values in group III.16 We also had few serum creatinine comparisons, where the samples were measured at different point of time with the samples drawn at the same time between the two methods. These values were included, as study by Loretta Ford et al17 concludes that delay in the separation of clotted blood samples have shown increase in creatinine is significant after only a 16-hour delay in sample separation.

We also had few samples which flags in creatinine results in ABG analyzer, which was not included in the above data and analyzed separately. This was analyzed, because in emergency conditions, these values were used for treatment. Hence the results of this would help the clinician for better decision when required. The mean difference in this group was 0.894 and ICC 0.795, where the mean difference is very high, and low correlation compared to the samples without flags. This guides the treating physician, to not consider the results which has flags.

CONCLUSION:

We found that the whole blood creatinine levels on the Radiometer ABL 800 FLEX without flags, has good agreement and good correlation with the serum creatinine level on the Architect Abbot ci8200 auto analyzer. The clinicians can use the results without flags from the point of care testing device by enzymatic method and no need to repeat the test by Jaffe's method. This leads to decrease in cost, early clinical decision and treatment of the patient in emergency department

References

1. Draczevski L, Teixeira L. Evaluation of per filbiochemical and parameters haematological in patients subjected to dialysis. Rev Saud Pesq. 2011; 4: 15-22.

2. Mascha V, Rashmi K, Priyadarshini SS, et al. Comparing age wise reference intervals for serum creatinine concentration in a "reality check" of the recommended cut-off. Indian J Clin Biochem. 2006; 21:90-94.

3. Jaffe M. Uber denniederschlag, welchen pikrinsaure in normalen hrn erzeugt und uber eineneue reaction des kreatinins. Z Physiol Chem 1886; 10:391-400.

4.   Wen-Sheng Liu, Yu-Ting Chung, Chih-Yu Yang, et al. Serum Creatinine Determined by Jaffe, Enzymatic Method, and Isotope Dilution-Liquid Chromatography-Mass Spectrometry in Patients Under Hemodialysis. Journal of Clinical Laboratory Analysis 2012; 26: 206–214.

5.   Creatinine. Hyderabad. M/s Excel Diagnostics Pvt. Ltd.

6.   Vaishya R, Arora S, Singh B and Mallika V. Modification of Jaffe's Kinetic Method Decreases Bilirubin Interference: A Preliminary Report. Indian Journal of Clinical Biochemistry, 2010; 25 (1) 64-66.

7.   Nichole L. Korpi-Steiner, Eric E. Williamson, and Brad S. Karon. Comparison of Three Whole Blood Creatinine Methods for Estimation of Glomerular Filtration Rate Before Radiographic Contrast Administration. Am J Clin Pathol 2009; 132: 920-926. DOI: 10.1309/AJCPTE5FEY0VCGOZ.

8.   Robert L. Schmidt, Joely A. Straseski, Kalani L. Raphael, et al. A Risk Assessment of the Jaffe vs Enzymatic Method for Creatinine Measurement in an Outpatient Population. PLOS ONE 2015. DOI: 10.1371/journal.pone.0143205

9.   Schweiger M, Chambers C, Davidson C, et al. Prevention of contrast induced nephropathy: recommendations for the high-risk patient undergoing cardiovascular procedures. Catheter Cardiovasc Interv. 2007; 69:135-140.

10.   Briguori C, Airoldi F, D'Andrea D, et al. Renal insufficiency following contrast media administration trial (REMEDIAL). Circulation. 2007; 115:1211-1217.

11.   Marakala V, Avinash S.S, Shivashankara A. R, et al. Serum creatinine assay: Enzymatic Vs Kinetic Jaffe's Method. Journal of Evolution of Medical and Dental Sciences. 2012; 1(4): 258.

12.   Moore J F, Sharer J D. Methods for Quantitative Creatinine Determination. Curr Protoc Hum Genet. 2017; 93(1):30.1-30.7.

13.   Korpi-Steiner NL, Williamson EE, Karon BS. Comparison of three whole blood creatinine methods for estimation of glomerular filtration rate before radiographic contrast administration. Am J Clin Pathol 2009; 132:920-6.

14.   Nichols JH, Bartholomew C, Bonzagi A, et al. Evaluation of the IRMA TRU point and i-STAT creatinine assays. Clin Chim Acta 2007; 377:201-5.

15.   Shephard M, Peake M, Corso O, et al. Assessment of the Nova Stat Sensor whole blood point-of-care creatinine analyser for the measurement of kidney function in screening for chronic kidney disease. Clin Chem Lab Med 2010; 48:1113-9.

16.   Anne-Sophie B, Nils Kuster, Etienne Cavalier, et al. Serum creatinine: advantages and pitfalls. J Lab Just Med 2018; 3:71.

17. Loretta Ford and Jonathan Berg. Delay in separating blood samples affects creatinine measurement using the Roche kinetic Jaffe method. Ann Clin Biochem 2008; 45: 83–87. DOI: 10.1258/acb.2007.007109.