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Evaluation and Comparison of Serum Creatinine Assay: Enzymatic Method Vs Kinetic Jaffe's Method

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ABSTRACT

The principal motive of this research is to measure the analytical performance and practicability of enzymatic method with Kinetic Jaffe's method. The cross sectional study was carried on 100 serum samples of outpatient population. In order to measure serum creatinine, with Beckman AU480 auto analyzer, transasia reagent was used in Kinetic Jaffe's method whereas coral reagent was used in enzymatic method. Pearson correlation coefficient method was applied to estimate the degree of association between two quantitative variables. The method comparison between the Enzymatic and Kinetic Jaffe's has shown regression coefficient of 0.99, which means both the methods correlate with each other. The intra assay and inter assay precision values were found to be within desirable level in both the methods. Hence, the experimental result had shown that both the methods of creatinine analysis were comparable with respect to performance and precision.

Keywords
Creatinine; Kinetic Jaffe's; Enzymatic Assay; Chronic Kidney disease

Introduction

In recent years, the overall degree and model of Chronic Kidney diseases has been studied periodically. Serum creatinine is a significant laboratory marker for renal function. The estimated Serum Creatinine measurement plays a vital role in glomerular filtration rate estimation (eGFR), Chronic Kidney disease diagnosis (CKD) as well as CKD treatments. The standardization of serum creatinine measurements is very important due to the central role of biomarker for the assessment of renal function. Therefore, an accurate measurement of serum creatinine is of utmost importance[1, 2, 3].

Though several methods have been recommended for estimating serum and urinary concentration of creatine, most of them were based on the Jaffe‟s reaction described first by Jaffe in 1886. Over the years, the Jaffe‟s assay has progressed through many phases [4]. There has been a major analytical problem associated with the use of the Jaffe‟s reaction, in particular to those relating to positive and negative interference by chromogens [5]. More than 50 chromogenic interfering substances have been documented. The commonly encountered interfering substances of the Jaffe‟s based methods include glucose, acetoacetate, bilirubin, and cefoxitin [6, 7].

The Jaffe's based serum creatinine method is very popular due to its simplicity and being economical. However, Enzymatic method tends to be superior to the Jaffe's method since it is dealt effectively with most interfering substances but is expensive compared to Jaffe's method. Though the Enzymatic method has a higher accuracy than another one, the evaluation and comparison of Serum Creatinine of Enzymatic and Kinetic Jaffe's methods are required to find the degree of association between the two quantitative variables. Hence, the study was conducted.

Methods and Materials

The study was conducted in the Department of Biochemistry of Stanley Medical College & Hospital (SMCH), Chennai. The request for approval of the study was obtained from the head of the department in a meeting held on 17.09.2018, SMCH. The cross sectional study was chosen for design and analysis. The total duration taken for this study was 6 months. The research was performed over 100 serum sample excluding certain criteria such as age <18

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& >60 years within the medical decision limit. Each sample was analyzed in duplicate by Enzymatic and Kinetic Jaffe's method. All measurements were performed using Beckman AU 480 auto analyser.

Method 1: Jaffe's Kinetic
Principle: Creatinine in alkaline solution reacts with picric acid to form a colored complex. The amount of complex formed is directly proportional to the creatinine concentration.

Creatinine + Alkaline Picrate $\longrightarrow$ Creatinine Picrate Complex

Method 2: Enzymatic

Principle: Creatinine + H_2O $\xrightarrow{\textbf{creatininase}}$ Creatine

Creatine + H_2O $\xrightarrow{\textbf{creatinase}}$ sarcosine + urea

Sarcosine + O_2 + H_2O $\xrightarrow{\textbf{sarcosine oxidase}}$ glycine + HCHO + H_2O_2

H_2O_2 + 4-aminophenazone $\xrightarrow{\textbf{peroxidase}}$ quinoneimine chromogen + H_2O

The color intensity of the quinone imine chromogen formed is directly proportional to the creatinine concentration.

Result Analysis and Discussions

The results of creatinine levels obtained from both methods were compared by using students paired t-test. While the mean of serum creatinine level by Jaffe's method (2.36±1.33), and enzymatic method (2.27±1.37) and the p value was 0.89 which is shown in table 1.

Table 1. Paired t –Test Comparison between Kinetic and Enzymatic

Samples	Method	Mean ±SD(mg/dl)	Mean Difference ± (mg/dl)	P Value
N=100	Enzymatic	2.27±1.37	-0.09±1.07	0.89
	Kinetic Jaffe's	2.36±1.33		

Table 2. Comparison by Precision Values

Within Run		
Method	Comparative (JAFFE)	Test (Enzymatic)
No. of Samples	100	100
Mean(mg/dl)	3.87	3.9
SD	0.07	0.11
CV%	1.8	2.8
Between Run		
Method	Comparative (JAFFE)	Test (Enzymatic)
No. of Samples	100	100
Mean(mg/dl)	3.83	3.95
SD	0.11	0.1
CV	2.8	2.5

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While the quality control analysis for within run precision by jaffe's method showed a mean 3.87, standard deviation (SD) 0.07, Correlation Variance (CV) 1.8, and a P value of 0.31, the precision by enzymatic method had shown a mean 3.90, SD 0.11, CV 2.8, and P value of 0.31. Likewise, the quality control analysis for between run precision by jaffe's method shown a mean 3.83, SD 0.11, CV 2.8, and P value of 0.00, while the quality control analysis for between run precision by enzymatic method had shown a mean of 3.95, SD 0.1, CV 2.5, and P value of 0.00.

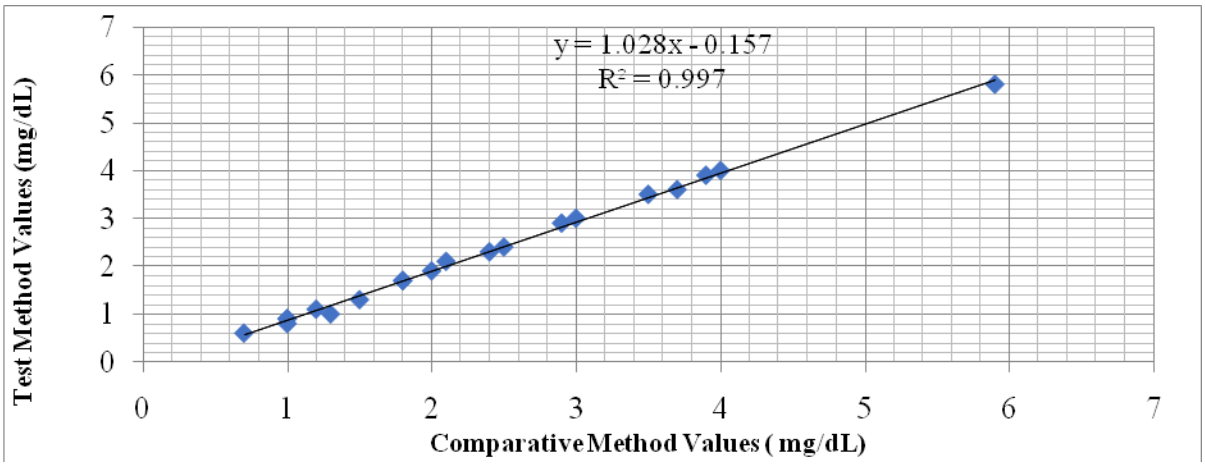

Figure 1. Linear Regression Analysis

The method comparison between Enzymatic (y) and Jaffe's (x) was performed by linear regression analysis y= 1.02x-0.157 and coefficient correlation (r =0.99). The result has no statistical difference and the same was consistent with the study of Marakala, et al [1, 4], while the serum creatinine measure was statistically significant for different group of patients[3].
Differently, the analysis between the semi-automated Jaffe methods with enzymatic method was not correlating each other; while the compensated Jaffes method with Enzymatic was no statistically significant [2].

Table 3. Statistical Analysis for Different Error Rates

Medical Limit Chosen	At 0.6mg/dl	At 6 mg/dl
Calculated RE by precision studies	8.4	8.4
Constant Systematic Error %	0.26	0.026
Proportionate Error %	2.8	1.6
Total Error	11.46	10.02

In table 3, While the total error was calculated by the formula TE= RE+ CE+ PE, the random error (RE) was calculated using the highest CV % from precision study. Likewise, while the constant systematic error (CE) was calculated by y-intercept from regression analysis, the proportional systematic error (PE) was calculated by slope of the regression analysis. In our study, the estimation of creatinine by enzymatic method showed no statistically significant mean difference (-0.09) with kinetic jaffe's method. The correlation coefficient of 0.99 was indicating a very good agreement between the two methods. While the intra assay and inter assay precision data were under desirable level in both the methods, the total error obtained at both medical decision limits was less than the total allowable error for creatinine (15%) as per CLIA criteria. Hence, in routine clinical care both the methods are recommended.

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Conclusion & Future Studies

The statistical analysis had shown clearly that both methods were comparable with respect to performance and precision. With large sample size, using recovery studies, sensitivity and specificity attributes the effect of interfering substances and validation of two methods needs to be analyzed in future. The accuracy and precision of the methods can be increased using external and internal quality control programs.

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