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Original Article

Study of Creatinine Estimation by Enzymatic and Kinetic Jaffe's Method

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Abstract

Background and Objectives: An enzymatic kit method for the determination of serum creatinine was optimized for use with Beckmancoulter 5800 Auto analyzer and its performance characteristics and practicability compared with kinetic Jaffe-based method. Effects of some common interfering substances like glucose and bilirubin on the kinetic Jaffe's and the enzymatic methods were compared. Method comparison between the enzymatic creatinine method (y) and Jaffe's kinetic method (x) gave the following equation for the normal group: y = 0.97*x+0.0 and a coefficient correlation R of 0.98.There was very good agreement between both the methods as intra class correlation coefficient (ICC) was between 0.81-1.

Method: We analysed 100 serum samples obtained for routine clinical care. Creatinine was analyzed both by kinetic Jaffe's and enzymatic method.

Results: Mean between enzymatic to kinetic Jaffe's methods were 1.105 mg/dl, 1.12. Overall mean difference between the two methods was 0.081 mg/dl.All of the above differences were statistically insignificant ($P > 0.05$). P value 0.44 which was insignificant.

Conclusion: In this study there was no statistically significance mean difference between both methods. The Intra class correlation coefficient between the two methods in, indicates a very good agreement between Kinetic Jaffe's method and enzymatic method. Hence in routine clinical care both the methods can be used.

Key words: Creatinine, Enzymatic assay, Kinetic Jaffe's

INTRODUCTION

Routine clinical biochemistry laboratories use several methods for the estimation of serum and urinary concentrations of creatinine, most of which are based on the Jaffe's reaction described first by Jaffe in 1886. Over the years, the Jaffe's assay has progressed through many phases. There are major analytical problems associated with the use of the Jaffe's reaction, in particular those relating to positive and negative interference by chromogens. More than 50 chromogenic interfering substances have been documented.[1] Commonly encountered interfering substances of the Jaffe's-based methods include glucose, acetoacetate, bilirubin, and cefoxitin.[2] Glucose and bilirubin both inhibit the reaction between creatinine and alkaline picrate. Glucose slowly reduces picric acid to picramate,[3] while bilirubin, under alkaline conditions, is oxidized to biliverdin, causing a decrease in absorbance at 520 nm.[4] Acetoacetate and cefoxitin, conversely, react directly with alkaline picrate and cause positive interference. Acetoacetate, in fact, reacts more rapidly with picrate than creatinine.[5] Enzymatic creatinine assay is widely accepted as one of the most accurate routine methods available at present. Several studies concluded that enzymatic method is suitable as a routine diagnostic laboratory method for the measurement of serum creatinine, particularly for diabetic ketotic patients, neonates, and patients receiving cephalosporins.[6] The enzymatic method exhibits several advantages over Jaffe's-based methods, namely, improved specificity smaller sample volume and hence a rapid sample throughput. Glucose, acetoacetate, and cefoxitin do not interfere with the enzymatic method, although bilirubin causes a negative interference which depends on both

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creatinine and bilirubin concentrations. The enzymatic creatinine assay deals effectively with most interfering substances but has a greater cost and shorter shelf-life compared with the kinetic Jaffe's method.[7] In this study, an enzymatic kit method for the determination of serum creatinine was optimized for use with Beckman Coulter AU 5800 autoanalyzer and its performance characteristics and practicability were compared with kinetic Jaffe-based method. The aim of this study was to compare analytical performance and practicability of the enzymatic method and kinetic method for serum creatinine for routine use and to compare the effects of some common interfering substances such as glucose and bilirubin on the enzymatic method and kinetic Jaffe's method.

MATERIALS AND METHODS

The present study was conducted in Osmania General Hospital, Department of Biochemistry, Hyderabad in 2020. We analyzed 100 serum samples obtained for routine clinical care. Creatinine was analyzed both by kinetic Jaffe's and enzymatic method. The Jaffe's method for serum creatinine determination is based on the principle that picric acid in an alkaline medium reacts with creatinine to form an orange-colored complex with the alkaline picrate. Intensity of the color formed during the fixed time is directly proportional to the amount of creatinine present in the sample. The enzymatic assay for creatinine involves a series of coupled enzymatic reactions including creatininase enzymatic conversion of creatinine into the product creatine which is converted to sarcosine by creatine amidinohydrolase (creatinase) followed by oxidation of sarcosine by sarcosine oxidase-producing hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide is quantified at 550 nm by the formation of a colored dye. All measurements were performed using an Beckman Coulter AU 5800 analyzer. The two levels of quality control materials used in this study were supplied from BioRad. We also estimated serum total bilirubin by azobilirubin method and fasting plasma glucose by hexokinase method of the respective subjects and the data comprising samples without interfering substances (samples having fasting plasma glucose <126 mg/dl and serum total bilirubin ≤1 mg/dl).

RESULTS

Mean between enzymatic to kinetic Jaffe's methods was 1.105 mg/dl, 1.12. Overall mean difference between the two methods was 0.081 mg/dl. All of the above differences were statistically insignificant ($P > 0.05$). *P*-value 0.44 was insignificant.

Estimation by two different method

Method	No. of sample	Mean±SD	Variance	*P*-value
Kinetic Jaffe's	100	1.12±0.21	0.043	0.44
Enzymatic	100	1.10±0.20	0.045	

DISCUSSION

The enzymatic method exhibits advantages over Jaffe's-based methods, namely, smaller sample volume (10 μL) and free of interference from substances such as glucose, acetoacetate, and bilirubin. The enzymatic technique yields result directly proportional to the kinetic Jaffe's reaction. Access to enzymatic assays can also be useful when interference from substances such as bilirubin and hemolysis is suspected.[8] On the other hand, a very few compounds may interfere with enzymatic procedures. Interference for enzymatic assays has been reported in case of intravenous fluid contamination of plasma samples from dopamine or dobutamine solutions.[9] The only drug reported to interfere with currently available enzymatic assays at borderline therapeutic concentrations is calcium dobesilate, used to reduce capillary permeability in diabetic retinopathy.[10] The enzymatic creatinine methods appear to be the only assays giving reliable results when specimens take time to reach the laboratory and blood centrifugation is delayed for 24 h or more. In a recently published study, delays in sample centrifugation caused false increases in measured creatinine by alkaline picrate assays due to the possible interference effect of some metabolites built up *in vitro*, such as pyruvate or ketones.[11] A minor disadvantage of the enzymatic method is its relatively high cost. In our study, estimation of creatinine by enzymatic method showed no statistically significant mean difference (–0.043) with the kinetic Jaffe's method, which is used by several laboratories (including our own center) in samples without glucose and bilirubin interference.

Method comparison between the enzymatic creatinine method (x) and kinetic Jaffe's method (y) gave the following equation for the whole group of 100 individuals: $y = 0.97*x–0.04$ and a correlation coefficient of 0.99. The creatinine kinetic Jaffe method gave substantially higher values compared with the enzymatic method. These results are in accordance with several studies that compared an enzymatic method with the kinetic Jaffe method. These results indicate that Jaffe methods, based on an alkaline picrate reaction, overestimate true serum creatinine concentrations due primarily to non-specific protein interference.[12-14]

In this study, there was no statistically significance mean difference between both methods. The intraclass

correlation coefficient between the two methods in, indicates a very good agreement between Kinetic Jaffe's method and enzymatic method. Hence, in routine clinical care, both the methods can be used.

Since the sample volume required is lesser, the throughput is higher, the interfering substances are fewer for the enzymatic method and since there is good agreement and good comparability with the kinetic Jaffe's method, the enzymatic method for estimation can be preferred, especially in the setting of neonates, diabetic, ketoacidosis, jaundice, and hemolytic samples. In accordance to ours, another study 19 evaluated 29 samples with bilirubin concentrations between 0.1 and 22.7 mg/dL (1.7–388.2 μmol/L) and did not find a significant difference between two methods of creatinine measurement (enzymatic [Ortho Vitros 950] and Jaffe's colorimetric on two different analyzers [Roche Hitachi 917 and Dade Dimension RXL]).

CONCLUSION

Enzymatic and kinetic Jaffe's methods of creatinine analysis were comparable with respect to performance in the presence and absence of interfering substances glucose and bilirubin, and imprecision. In this study, we employed a small sample size and could test effects of only two interfering substances. Future studies will aim at analyzing the effects of many more interfering substances and validation of two methods by recovery studies, analysis of accuracy, sensitivity and specificity, and evaluation of the methods under allowable imprecision level. Both external and internal quality control programs will be utilized to increase the accuracy and precision of the creatinine assay methods.

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