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COMPARISION OF SERUM CREATININE ESTIMATION BY ENZYMATIC VERSUS JAFFES KINETIC METHOD

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ABSTRACT

Background: Creatinine is an anhydrous form of creatine. Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a constant rate by the body. Measurement of creatinine requires accuracy as it is an important biomarker which reflects renal dysfunction. There is debate regarding its measurement as we have popular jaffes kinetic method and enzymatic method. **Aim and Objectives:** To measure the serum levels of serum creatinine in subjects with renal dysfunction and healthy controls by Jaffes method and Enzymatic method. **Materials and Methods:** we included a total of 240 samples which were divided into 6 groups based on the levels of serum creatinine, serum bilirubin, fasting plasma glucose and combination of elevated plasma glucose and bilirubin levels. **Discussion and Conclusion:** This study has indicated that Jaffe and kinetic methods show poor agreement for the estimation of serum creatinine in the presence of renal dysfunction. Serum creatinine is underestimated by Modified Jaffes Kinetic method in the presence of bilirubin and overestimated in the presence of glucose. Serum creatinine is underestimated by Modified Jaffes Kinetic method in the presence of both bilirubin and glucose. When Serum Creatinine level is >5 mg/dl, it is underestimated by Modified Jaffes Kinetic method. Enzymatic has greater linearity than the Modified Jaffes Kinetic Method. Enzymatic method is less affected by interferences so it is a better method to measure creatinine. The enzymatic method is more reliable when interfering substances are present in the samples analysed, which makes a method of choice.

KEYWORDS : creatinine, bilirubin, jaffes kinetic method, enzymatic method and plasma glucose.

INTRODUCTION:

Creatinine is an anhydrous form of creatine. Creatinine is a breakdown product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body [1]. This muscle metabolite serves as an important indicator of renal function since it is excreted largely unchanged by the kidneys [2].

A reliable estimate of renal function can be made by comparing blood urea nitrogen (BUN) and creatinine values obtained per unit time from the same individual [2]. Estimation of serum creatinine is estimated by Jaffes end-point method and also by enzymatic method.

The Jaffe's endpoint method was described by Max Jaffe in 1886 for creatinine assay. This method has since been the most widely and commonly used for creatinine assays. The Jaffe reaction is not specific for creatinine as many compounds have been reported to produce a Jaffe-like chromogen, including protein, glucose, ascorbic acid, bilirubin, ketone bodies, pyruvate, guanidine, haemoglobin F, and blood- substitute products [3]. Moreover, this Jaffe endpoint is time consuming and not readily automated [4].

In the kinetic Jaffe method, is based on alkaline picrate. At an alkaline pH creatinine in the sample reacts with picrate to form creatinine-picrate complex. The rate of increase in absorbance at 500 nm because of this complex is directly proportional to the concentration of creatinine in the sample [5]. Although this method eliminates some of the nonspecific reactants, it is subject to interference by α-keto acids and cephalosporins (Bowers and Wong, 1980) [6]. Bilirubin and hemoglobin may cause a negative bias, probably a result of their destruction in the strong base used. The kinetic Jaffe method is used routinely despite these problems because it is inexpensive, rapid, and easy to perform [7].

The other method for estimation of creatinine is enzymatic method. The principle of enzymatic method is Creatinine in the sample is hydrolyzed by creatinase to sarcosine and urea. Sarcosine from this reaction is oxidized by sarcosine oxidase to glycine and formaldehyde with concomitant production of H2O2. The H2O2 reacts with 4-amino antipyrine and N-ethyl-N-sulfoprophyl-m-toludine in presence of peroxidase to yield quinoneimina dye. The resulting change in absorbance at 548nm is proportional to the concentration of creatinine in the sample. In our clinical laboratory, routine creatinine analysis is performed on automated analyzer based on Jaffes method. In addition, we introduced enzymatic analysis of creatinine hence we have taken up this study to determine and compare the analytical performances by two methods for the estimation of creatinine levels in serum.

AIM AND OBJECTIVES: To measure the serum levels of serum creatinine in subjects with renal dysfunction and healthy controls by Jaffes method and Enzymatic method.

MATERIALS AND METHODS:

Site: This present study was conducted at RKDF Medical College, Dept. of Biochemistry Bhopal, MP from July 2022 to November 2022.

Study population: we included a total of 240 samples which were divided into 6 groups based on the levels of serum creatinine, serum bilirubin, fasting plasma glucose and combination of elevated serum glucose and bilirubin levels.

Group 1: Serum Creatinine 0.7-1.4mg/dL, number of subjects = 40
Group 2: Serum Creatinine 1.4-5 mg/dL, number of subjects = 40
Group 3: Serum Creatinine >5 mg/dL, number of subjects = 40
Group 4: Fasting serum glucose >126 mg/dL, number of subjects = 40
Group 5: Serum bilirubin >1 mg/dL, number of subjects = 40
Group 6: Serum bilirubin >1 mg/dL & FPG >126 mg/dL, number of subjects = 40

Study design: cross-sectional study.

Sample size: we included a total of 240 samples based on inclusion and exclusion criteria.

Exclusion criteria: we excluded haemolysed samples and lipemic samples.

Laboratory investigations: 2 mL of fasting venous blood samples were collected from each patient into plain tube and fluoride tube allowed to clot and centrifuged the sample for the measurement of the following parameters

1) Creatinine: by Jaffes and Enzymatic method.
2) Fasting plasma glucose: GOD-POD method.
3) Serum Bilirubin: Diazo method.

Statistical Analysis: Statistical analysis was done using Microsoft Excel spreadsheet, and statistical package for the social sciences (SPSS) version 20.0 software. The data was analysed and expressed as mean ± standard deviation. Statistical significance was assessed using student t test and the value of p was calculated. A p value <0.05 is considered. statistically significant.

This cross-sectional study was conducted in the Department of

Biochemistry.

RESULTS:

This cross-sectional study was conducted in the Department of Biochemistry, we included a total of 240 subjects.

Table 1: Shows the comparison of creatinine levels in groups by Enzymatic and Jaffes Kinetic methods

Groups	Enzymatic method	Jaffes Kinetic method	p-value
Group 1 (n = 40) S Creatinine 0.7-1.4 mg/dL	1.2 ± 0.78	1.1 ± 0.88	HS
Group 2 (n = 40) S Creatinine 1.4 - 5 mg/dL	2.68 ± 0.72	2.57 ± 0.62	HS
Group 3 (n = 40) S Creatinine > 5 mg/dL	8.68 ± 0.77	7.82 ± 0.65	HS
Group 4 (n = 40) Fasting serum glucose >126 mg/dL	1.44 ± 0.58	1.58 ± 0.72	HS
Group 5 (n = 40) Serum bilirubin >1mg/dL	1.61 ± 1.00	1.38 ± 0.99	HS
Group 6 (n =40) FSG >126 mg/dL + S. Bilirubin > 1mg/dL	2.68 ± 0.72	2.61 ± 0.69	S

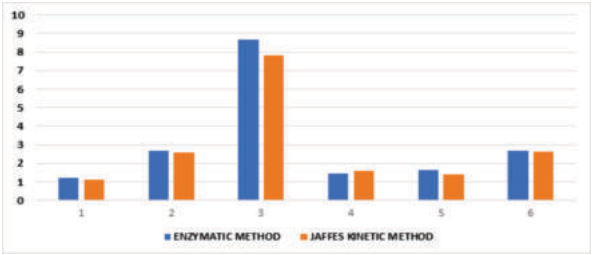

Figure 1: Shows the comparison of creatinine levels in groups by Enzymatic and Jaffes Kinetic methods

DISCUSSION:

In the present study, we included a total of 240 subjects, we divided the total subjects into six groups depending on the concentration of serum creatinine, fasting plasma glucose, and serum bilirubin. We measured serum creatinine by Jaffes method and enzymatic method.

The enzymatic method exhibits advantages over Modified Jaffes Kinetic (25µL) based methods namely, smaller sample volume (10µL) and free of interference from substances such as glucose and bilirubin. The enzymatic technique yields results directly proportional to the Modified Jaffes Kinetic reaction. Access to enzymatic assays can also be useful when interference from substances such as bilirubin and glucose is suspected.

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. A minor disadvantage of the enzymatic method is its relatively high cost.

Various studies have reported that there is no statistical significant "p" value in normal healthy individuals. Study of Vijaya Marakala et al reported no statistical significant "p" value 0.565 in normal healthy individuals. Irena I. Gencheva and Adelaida L. Ruseva reported no statistical significant "p" value >0.10 in normal healthy individuals [8, 9]. The results of our study were not in concordance with their finding. In case of high creatinine (Creatinine >1.4 to 5 mg/dl) by enzymatic method showed statistically significant "p" value, HS with the Modified Jaffe□s kinetic method in samples without glucose and bilirubin interference. In case of high creatinine (Creatinine > 5 mg/dl) by enzymatic method showed statistically significant "p" value, HS with the Modified Jaffes kinetic method in samples without glucose and bilirubin interference. In the presence of glucose interference (glucose > 126 mg/dl), the samples showed statistical significant "p" value, HS between enzymatic and Modified Jaffes kinetic method. However, it is important to note that there was a poor agreement between Jaffe and kinetic methods. This may not be unrelated with these cofounders making it difficult to interpret renal functions results. The results of our study are in agreement with other studies [10,11,12].

CONCLUSION:

This study has indicated that Jaffe and kinetic methods show poor agreement for the estimation of serum creatinine in the presence of renal dysfunction. Serum creatinine is underestimated by Modified Jaffe□s Kinetic method in the presence of bilirubin and overestimated in the presence of glucose. Serum creatinine is underestimated by Modified Jaffe□s□Kinetic method in the presence of both□bilirubin and glucose. When Serum Creatinine level is >5 mg/dl, it is underestimated by Modified Jaffe□s Kinetic method.□ Enzymatic has greater linearity than the Modified Jaffe□s Kinetic Method. Enzymatic method is less affected by interferences so it is a better method to□ measure creatinine.□ The enzymatic method is more reliable when interfering substances are present in□ the samples analyzed, which makes a□method of choice.

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LIMITATIONS: The sample size in our study was less, larger sample size is needed to support our findings.

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