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Evaluation of an Enzymatic Method for Determining Serum Creatinine Assay

KEYWORDS: Creatinine , Enzymatic Assay, Jaffe's Kinetic Assay, Bilirubin , Glucose

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ABSTRACT The enzymatic method for the determination of serum creatinine is accepted as one of the standard method in a clinical laboratory. The enzymatic method for the determination of serum creatinine was optimized for use with Merilyzer AutoQuant-400 auto analyzer and its performance characteristics were practically compared with Jaffe's Kinetic. Effects of some interfering substances like Serum bilirubin and plasma glucose on the Jaffe's kinetic method and the enzymatic method were compared. We measured creatinine in serum samples with the enzymatic method and the Jaffe's kinetic method in samples divided four groups; Group I-Samples without bilirubin and glucose; Group II-Sample with high level of plasma glucose ; Group III-Samples with high level of bilirubin and Group IV-all the samples. There was an excellent agreement between the two methods in terms of correlation coefficient even in the samples with high levels of glucose or bilirubin. Enzymatic method is having better sensitivity, less interfering effects & having better choice in making decision of critical management of the renal failure patient.

Introduction:
The creatinine determination in clinical practice is more than 100 years old, there is still much debate regarding its accuracy. Also, numerous methods have been described for determining creatinine in biological fluids. Many of the currently need procedures are based on the Jaffe's alkaline picrate procedure, which is not specific and is subject to interferences [1]. Commonly encountered interfering substances of the Jaffe's based methods include glucose, acetoacetate, bilirubin and Cefoxitin (Cephalosporins)[2]. Glucose slowly reduces picric acid to picramate, while bilirubin, under alkaline condition, is oxidized to biliverdin, causing a decrease in absorbance at 520 nm. Acetoacetate and Cefoxitin, conversely react directly with alkaline picrate and cause positive interferences. Acetoacetate , in fact reacts rapidly with picrate than creatinine[3]. Many investigators have attempted to improve the procedure by minimizing the effect of interfering substances present in the sample. Enzymatic approached have been used, to increase specificity. 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 negative interferences which depends on both creatinine and bilirubin concentrations.

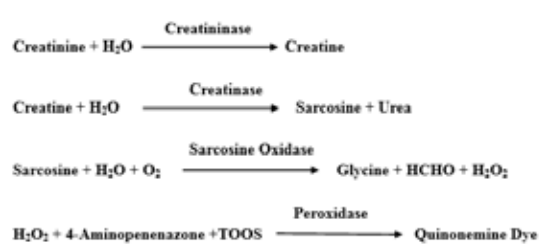
The aims of this study was to compare analytical performance and practicability of the enzymatic method and kinetic method for serum creatinine for routine use and compare the effects of some common interfering substances like glucose and bilirubin on the enzymatic method and kinetic Jaffe's method.

Materials and Methods:
The Present study was conducted in the clinical biochemistry Laboratory. We assessed 512 consecutive serum samples collected for routine clinical case.

Creatinine was analyzed both the Jaffe's kinetic and the enzymatic method. The Jaffe's method of serum creatinine determination in based on the principle that picric acid in an alkaline reacts with creatinine to form a yellow-red complex with the alkaline picrate[4]. Intensity of the colour 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 creatinine which is converted to sarcosine by creatine amido hydrolase (creatinase), followed by oxidation of sarcosine by sacrosine oxidase producing hydrogen peroxide.

In the presence of peroxidase the hydrogen peroxide is quantified at 550 nm by the formation of Coloured dye[2]. All Measurements were performed using Merilyzer Auto-Quant-400 auto analyzer. We also, estimated serum total bilirubin by Diazo method and plasma glucose by hexokinase method of the respective samples.



The two levels (normal and pathological) of quality control, materials used in this study were supplied from BioRad.

The data obtained were divided into three groups. Group I - comprised 167 samples without interfering substances (samples with plasma glucose <126 mg/dl and serum total bilirubin 1 mg/dl) Group II comprised 206 samples with bilirubin (samples with serum total bilirubin< 1.0 mg/dl and plasma glucose < 126 mg/dl); Group III comprised 139 samples with plasma glucose (Plasma glucose >126 mg/dl and serum total bilirubin <1.0 mg/dl, and Group IV all 512 samples.

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We determined the mean difference between the two methods and analyzed the agreement between them. The two methods were also compared by regression analysis. The Two levels of interval quality control materials were analyzed for enzymatic and Jaffe's kinetic methods.

We determined the mean difference between the two methods and analyzed the agreement between them. The Two levels of internal quality control materials were analyzed for enzymatic and Jaffe's kinetic methods. Statistical Package (SPSS, version 11.0) was used for statistical analysis. After analyzing data obtained using two methods, the significance of differences between the methods was determined. Linear regression model was used to establish correlation coefficients.

Results:

In this study, estimation of creatinine by enzymatic method showed statistically significant p-value with the Jaffe's method, except in the normal group. In the presence of high glucose level, results from sample analyses showed no statistical significant difference between enzymatic and Jaffe's kinetic method. When bilirubin was present in the serum samples, the differences enzymatic and Kinetic Jaffe's method was also statistically insignificant, represented in Table 1.

Group	Method (No of Sample)	Mean Value ± SD in mg/dl	p-Value
Group I	Enzymatic (n = 167)	1.17 ± 0.95	0.64
	Jaffe's Kinetic (n = 167)	1.22 ± 0.99	
Group II (Bilirubin)	Enzymatic (n = 216)	1.22 ± 0.47	0.0025
	Jaffe's Kinetic (n = 216)	1.37 ± 0.55	
Group III (Glucose)	Enzymatic (n = 129)	1.49 ± 1.52	0.44
	Jaffe's Kinetic (n = 129)	1.64 ± 1.59	
Group IV	Enzymatic (n = 512)	1.29 ± 0.98	0.058
	Jaffe's Kinetic (n = 512)	1.41 ± 1.04	

p<0.005 (p- values for mean difference between two methods by student't' test)

This table-1 shows estimated parameters Mean, SD and p-value. In Group I (normal), p-Value is > 0.64. In the second group (group II) (high bilirubin), p-value is considered to be very statistically significant. ($p \leq 0.0025$. In Group III and Group IV, p-values were not considered to be not quite statistically significant.

Also, correlation coefficient for both methods in all form groups is shown on figure1-4.

The quality control analysis of level -I for precision by enzymatic method (n= 20) yielded a mean, SD and CV% depicted in table 2 and 3.

Harmonization Studies:

Table 2: Method Comparison Harmonization Studies between Jaffae's Kinetic and Enzymatic Method (Level 1)

Creatinine Level 1	Jaffae's Kinetic	Enzymatic	Average	Difference
	1.65	1.5	1.575	0.15
	1.63	1.58	1.605	0.05
	1.65	1.60	1.625	0.05
	1.66	1.53	1.595	0.13
	1.62	1.63	1.625	-0.01
	1.58	1.5	1.54	0.08
	1.62	1.66	1.64	-0.04
	1.6	1.63	1.625	-0.03
	1.57	1.59	1.58	-0.02
	1.6	1.58	1.59	0.02
	1.54	1.57	1.555	-0.03
	1.57	1.59	1.58	-0.02
	1.6	1.52	1.56	0.08
	1.55	1.58	1.565	-0.03
	1.59	1.60	1.595	-0.01
	1.60	1.63	1.615	-0.03
	1.63	1.58	1.605	0.05
	1.66	1.6	1.63	0.06
	1.64	1.54	1.59	0.1
	1.63	1.59	1.61	0.04

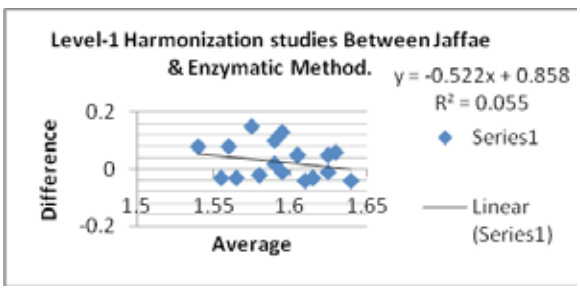

Fig: 1

Table 3: Method Comparison Harmonization Studies between Jaffae's Kinetic and Enzymatic Method (Level 2)

Creatinine-Level-2	Jaffae's Kinetic	Enzymatic	Average	Difference
	5.87	5.23	5.55	0.64
	5.93	5.29	5.61	0.64
	6.1	5.3	5.7	0.8
	6.03	5.28	5.655	0.75
	5.92	5.31	5.615	0.61
	5.87	5.2	5.535	0.67
	5.81	5.28	5.545	0.53
	5.93	5.3	5.615	0.63
	5.7	5.29	5.495	0.41
	5.66	5.24	5.5	0.32
	5.80	5.27	5.535	0.53
	5.9	5.26	5.58	0.64
	5.98	5.32	5.65	0.66
	5.89	5.36	5.625	0.53
	5.89	5.31	5.6	0.58
	5.89	5.26	5.575	0.63
	5.83	5.31	5.57	0.52
	5.75	5.29	5.52	0.46
	5.72	5.37	5.545	0.35
	5.78	5.30	5.54	0.48

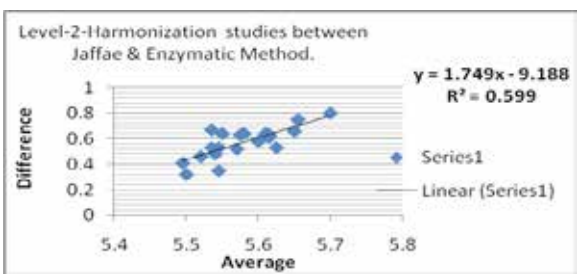

Fig: 2

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Harmonization studies were undertaken in Creatinine measurements by using two levels of commercial controls having different concentrations. Regression analysis shows that majority of data points of both controls(Level 1 and Level 2) are homo sadistically distributed along the regression line indicating close agreement between the two methods having maximum deviations within 0.1 mg/dl in level1 & 0.8 mg/dl in level2 controls represented in the Tables 2 and 3 & Fig 1 and Fig 2.

Linearity Studies:
It was undertaken by using serial dilutions of Patient's serum having higher creatinine concentrations & slight turbidity. Measurement were undertaken in same sample by using both Jaffae's Kinetic & Enzymatic methods reveals better sensitivity & less interfering effects ,represented in the table 4 and 5 & Fig 3 and 4.

Table-4

Alkaline Jaffae Method	Designated Value-mg/dl	Observed Response mg/dl
	17	17
	8.5	8.32
	4.25	4.30
	2.12	2.20
	1.06	1.16
	0.53	0.49
	0.265	0.30
	0.138	0.15

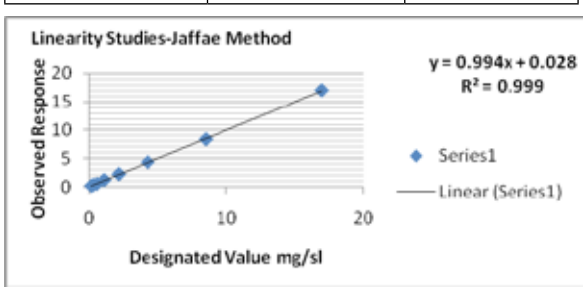


Fig: 3

Table-5

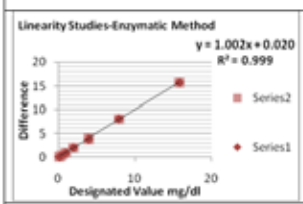
Enzymatic Method	Designated Value-mg/dl	Observed Response-mg/dl
	15.8	15.8
	3.95	3.89
	1.97	2.1
	0.98	0.96
	0.49	0.50
	0.246	0.23
	0.123	0.130

Fig: 4

Discussion:
The enzymatic method was found to have certain advantages over Jaffe's kinetic method, and especially lack of interference with substances such as glucose and bilirubin. The enzymatic technique yields results directly proportional to the kinetic Jaffe's reaction. Use of Jaffe's kinetic method yielded substantially higher values for creatinine, as compared with those obtained using the enzymatic method. These results indicate that Jaffe's kinetic methods, based on an alkaline picrate reaction, over estimate true serum creatinine concentration, mainly due to non-specific interference.

A very few compounds may interfere with enzymatic procedure. Interference for enzymatic assay has been reported in case of inter venous fluid concentration of plasma samples from dopamine or dobutamine solutions[5]. 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 hours or more. In a recently published study, delays in sample centrifugation caused false increases in measured creatinine by alkaline picrate assay due to the possible interference effect of some metabolites built up in vitro, such as pyruvate or ketones[6].A minor disadvantages of the enzymatic method is its relatively higher cost.

In our study, the correlation coefficient for the two methods in Group I ,Group II, Group III and Group IV (all samples),indicated a very good agreement between Jaffe's kinetic method and Enzymatic method(Table 2).

The results obtained proved a very good comparability between the two methods in all the settings specified, with or without the presence of interfering substances like glucose and bilirubin as well as comprising all the samples. The creatinine Jaffe's kinetic method has substantially higher values compared with the enzymatic method. The results are in accordance with several studies that compared an enzymatic method with the Jaffe's kinetic method. The results indicate that Jaffe's kinetic method, based on an alkaline picrate reaction, over estimate true serum creatinine concentration due primarily to non-specific protein interference[7].

In our study there was no statistically significance mean difference between both methods in all the groups and the difference was also not clinically significant.

The intra class correlation coefficient between the two methods indicates a very good agreement between Jaffe's kinetic method and enzymatic method. So routine clinical care both the methods can be used. Both the methods showed significant correlation with or without the presence of interfering substances (Table 1).

Replacing the Jaffe's kinetic method into routine laboratory practice is accordance with recent recommendation of the laboratory working Group of the National kidney Disease Education Programme[8]. This group suggests that the estimated glomerular filtration rate has to be reported using accurate and specific serum creatinine measurements, based on the concept of tracebility[9].

The interfering substances are fewer for the enzymatic method and since there is good agreement and good comparability with the Jaffe's kinetic method and enzymatic method for estimation can be preferred especially in the setting of neonates, diabetic, keto acidosis, jaundice and hemolytic samples.

The enzymatic method for creatinine evaluated in this study showed considerable improvement on the specificity

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of the existing Jaffe-based methods, although a problem still exists with interference from glucose and bilirubin. The modified enzymatic method was both precise and accurate, used a small sample size and was capable of a rapid throughput of patient's sample. Bilirubin interference in the enzymatic method can be eliminated by the addition of ferrocyanide to the reaction mixture, which stabilizes the reaction intermediate. We conclude that the enzymatic method is suitable as a routine diagnostic laboratory method for the measurement of serum/plasma creatinine, particularly for diabetic patients.

Conclusion:

In conclusion, enzymatic of creatinine analysis were comparable with respect to performance in the presence and absence of interfering substances glucose, bilirubin and imprecision.

Method comparison studies by regression analysis and linearity studies reveal the following facts: 1) Creatinine value is having higher trends in comparison to enzymatic method. 2) Enzymatic method is having better sensitivity, less interfering effects & having better choice in making decision of critical management of the renal failure patient. However, the enzymatic method is more reliable when interfering substances are present in the sample analyzed, which makes in a method of choice.

Acknowledgement

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