

Comparison between Jaffe and Enzymatic Creatinine Assays in Renal Dysfunction Subjects

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Abstract

False creatinine results estimated by conventional Jaffe's endpoints are still a problem in the laboratories. We hypothesized that delay in absorbance measurement may influence the concentration of creatinine measured by Jaffe's conventional method. This study compared the agreements between creatinine measured by conventional Jaffe's endpoints and kinetic Jaffe's methods and the influence of delayed absorbance reading on creatinine concentrations in kidney disease patients. A cross sectional study conducted in one hundred (70 males and 30 females) adults with kidney dysfunction. Serum creatinine was measured by Jaffe endpoint method and by Jaffe's Kinetic method. Significant increase was observed in the creatinine measured by Jaffe's endpoint method compared with Kinetic method. The serum creatinine concentration measured by Jaffe's endpoint methods increases with increase time. This study has indicated that conventional Jaffe endpoint and kinetic methods show poor agreement for the estimation of serum creatinine in the presence of renal dysfunction, the kinetic method performed slightly better. This study also suggests that delay in absorbance reading for Jaffe endpoint reaction is a factor which influences serum creatinine concentrations.

Keywords: Time, serum creatinine, end-points Jaffe's and Jaffe's kinetic methods.

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INTRODUCTION

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]. 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, serum is mixed with alkaline picrate and the rate of change in absorbance is measured [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].

Several researchers have conducted similar studies with conflicting results. Yet the conventional Jaffe's endpoint method is still widely employed as the method of choice for serum creatinine, despite its shortcoming of non specificity for creatinine and other interfering substances reportedly to cause falsely elevated creatinine results. Besides, we hypothesized that delay in absorbance measurement may influence the concentration of creatinine measured by Jaffe's conventional method.

MATERIALS AND METHODS

Ethical approval for this study was obtained from Research and Ethical Committee of Ministry of

Health, Sokoto State. Written and informed consent was sought and obtain from each of the participants with explanation on the aim and objectives of the research. Patients without kidney disease were excluded from the study. This will be a cross sectional study design which involved One Hundred Kidney diseased patients on treatment/medication for serum creatinine estimation by Jaffe’s Reaction. Each Kidney disease specimen was subjected to the following methods for serum creatinine estimation: Jaffe’s conventional method and Kinetic method. The duration for the study was two months in which five specimens were collected per day.

Two (2) ml of venous blood sample was collected into a plain container using a disposal plastic syringe from antecubital vein after which the blood was allowed to clot, and centrifuged at 4000 rpm for 5 minutes. The serum was harvested into a vial and analyzed for serum creatinine using Jaffe’s Reaction Method (Kinetic and endpoint). The quality control measures adopted for the study was pilot study, precision study and the use of standards of reagent. Data obtained from the study were analyzed with Graph Pad Prism software v. 3.1. Normality testing was done to check error in the data and to lead the choice of appropriate statistical method. The error in our data was

not normally distributed. Mann Whitney U was used for methods comparison and the level of agreement between the two methods was studied using Kappa Statistics. Friedman test was used to determine and compare the changes on the creatinine concentrations due to delayed absorbance measurement by conventional Jaffe’s endpoint method. P-values of less than 0.05 were considered as significant.

RESULTS

Serum creatinine levels measured by the Jaffe endpoint method was significantly (P<0.05) higher than kinetic endpoint method among renal dysfunction subjects, (Table 1). With respect to the Jaffe endpoints, the level of serum creatinine of the two groups (15 min and 30 min) differ significantly (P< 0.05) higher in comparison with immediate absorbance reading (Table 2). The correlation showed strong and statistically significant difference between the Jaffe and Kinetic methods. The agreement between Jaffe and Kinetic creatinine methods in the sample as a whole is weak and statistically insignificant (Table 3). A good experimental results with good inter and intra assay coefficient of variance between the creatinine methods (Table 4).

Table-1: Method variation of serum creatinine measurements among study participants

Parameters	Creatinine methods		U	Z-score	p-value
	Jaffe (n=100)	Kinetic (n=100)			
Creatinine (mg/dL)	1.63±0.15	1.40±0.14	2879.5	-2.291	0.022 (s)

Data are presented as mean ± SEM. U=Mann-Whitney test value.

Table -2: Influence of different time on Jaffe creatinine absorbance among study participants

Serum Creatinine (mmol/L)					
Timing	n	Sum of Ranks	FrS	p-value	
Group 1	100	108.00 ^a	116.80	0.0001 (s)	
Group 2	100	166.50 ^b			
Group 3	100	235.50 ^c			

Values with different superscript (^{a b c}) differ significantly (p<0.05) in comparison with each time. FrS=Friedman Statistics, n=observations. Group1=immediate absorbance. Group2= Absorbance after 15 minutes. Group3=Absorbance after 30 minutes.

Table-3: Correlation and reliability analysis of creatinine methods among study subjects

Jaffe Vs Kinetics methods		
Variables	Values	p-value
r-value	+0.801	0.001 (s)
kS-value	0.004	0.868 (ns)

r=correlation coefficient. +(positive correlation). KS=kappa statistics

Table-4: Precision study of the Jaffe and Kinetic methods for serum creatinine (SCr)

Co-efficient of variation (%)		
Methods	Inter assay CV (%)	Intra assay CV (%)
Jaffe method	90.0	3.89
Kinetic method	93.1	
Average CV	90.15	-

Data expressed as mean ± SEM. SCr=serum creatinine. %=percent. CV= Co-efficient of variation.

DISCUSSION

The serum creatinine concentration obtained showed that there was significantly lower serum creatinine with the kinetic method of estimation than with Jaffe's endpoint methods implying that kinetic Jaffe's method is better. This is not unexpected as Jaffe's endpoints method has been implicated with several interferences which are confounding variables which are a challenge till date for the specificity of creatinine assay and standardization across different laboratories. These confounding variables that are known to produce Jaffe-like chromogens include protein, glucose, ascorbic acid, ketone bodies, pyruvate, guanidine, cephalosporin and bilirubin [8], with protein having the greatest influence [9].

Further light has been thrown on this when looking at method performance that kinetic Jaffe reaction is better than endpoint Jaffe reaction in accuracy of results, short turnaround time, non-specificity, cost and interference by a large number of compounds [3]. Our data confirm these observations.

The influence of time on serum creatinine concentration by Jaffe's endpoint methods was also studied. The serum creatinine concentration increases with increase time. This is supported by extremely significance difference observed in the serum creatinine concentration between the immediate absorbance (T0) and absorbance after 15 minutes (T15) as well as T0 and absorbance after 30 minutes (T30). The difference in time in which endpoint Jaffe reaction takes longer time due to the multiple procedural steps which allow the interaction of interfering substances and increases the percentage of error and occurrence of false results while in kinetic Jaffe reaction there is no time problem because of good procedural advantage for absorbance reading immediately after 30 seconds, one minute and two minutes to overcome the effect of interfering substances and there is no time for consuming and less procedural steps which minimize the error [7]. These suggest further that the kinetic Jaffe method performed better and is preferred due to its improved turnaround time especially in referral centres with bulk of specimens for serum creatinine estimations. Further work will need to be done to authenticate this.

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.

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,

the kinetic method performed slightly better and also suggest that delay in absorbance reading for Jaffe endpoint reaction is a factor which influences serum creatinine concentrations.

RECOMMENDATIONS

It is suggested that creatinine concentrations should be read on time to avoid erroneous results in the Jaffe's method. A further study should be done to see the most suitable method for serum creatinine estimation by using reference methods with large sample size.

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