

American Journal of Advances in Medical Science
www.arnaca.com
Vol-2: No-1: 12-17: 2014

Original Research Article

A comparative estimation of plasma creatinine concentration using Jaffe's reaction and kinetic method in patients with chronic renal failure

Mohammed A. Mahdi¹, Gad Allah Modawe², Mohammed Amanullah^{3*}, Mohamed Babiker Abd Elrouf⁴, Gaffar Sarwar Zaman⁵

¹University of Medical Sciences and Technology, Faculty of Medical Laboratory Sciences, Department of Clinical Chemistry, Khartoum, Sudan.

²Omdurman Islamic University, Faculty of Medicine, Biochemistry Department, Omdurman, Sudan.

^{3,4,5}King Khalid University, College of Medicine, Department of Clinical Biochemistry, Abha, Kingdom of Saudi Arabia.

Abstract

Chronic Kidney Disease (CKD) is a clinical syndrome that occurs when there is a gradual decline in renal function over time. Creatinine (Molecular Weight 113 Dalton-MW 113 D) is the cyclic anhydride of creatine that is produced as the final product of decomposition of phosphocreatine. In the present work an attempt has been made to compare between results of plasma creatinine using endpoint Jaffe reaction and kinetic method in patients with chronic renal failure. Results from the present study conclude that kinetic Jaffe reaction is better than endpoint Jaffe reaction.

Key words: Plasma creatinine, renal failure, kinetic Jaffe reaction ,end point Jaffe

1.0 Introduction:

Failure of renal function may occur rapidly, producing the syndrome of acute renal failure. This is potentially reversible, if the patient survives the acute illness, normal renal function can be regained. However, chronic renal failure (CRF) develops insidiously, often over many years, and is irreversible, leading eventually to end-stage renal failure [1]. Acute renal failure is a sudden and sharp decline in renal function as a result of an acute toxic or hypoxic insult to the kidneys, defined as occurring when the Glomerular Filtration Rate (GFR) is reduced to less than 10ml/minute [1] or GFR less than 60 ml/min/1.73m² for at least 3 months [2]. It is characterized by a rapid loss of renal function, with retention of urea, creatinine, hydrogen ions and other metabolic products, and usually but not always, oliguria (<400ml urine/24h) and often develops in patients who are already severely ill. It is conventionally divided into three categories, according to whether renal functional impairment is related to a decrease in renal blood flow (pre-renal), to intrinsic damage to the kidneys (intrinsic), or to urinary tract obstruction (post-renal) [3]. Some of the causes of CKD are renal circulatory diseases, primary glomerular disease, renal sequelae to metabolic disease, inflammatory diseases, renal obstructions, congenital renal deformity and miscellaneous conditions.

Glomerular Filtration Rate (GFR) is the volume of plasma filtered (V) by the glomerulus per unit time (t) [1] and also clearance of a substance is the volume of plasma from which that substance is completely cleared by the kidneys per unit time [3]. Creatinine (Molecular Weight 113 Dalton-MW 113 D) is the cyclic anhydride of creatine that is produced as the final product of decomposition of phosphocreatine. Creatine is synthesized in the kidneys, liver, and pancreas [3] primarily in the liver from arginine, glycine, and methionine and then transported to other tissues, such as muscle, where it is converted to phosphocreatine, which serve as high-energy source. Creatine phosphate loses phosphoric acid and creatine loses water to form creatinine, which passes into the plasma.

Plasma creatinine concentration is a function of relative muscle mass and renal function [1]. Plasma creatinine remains within the reference interval until significant renal function has been lost. Complications of CKD include cardiovascular disease, bone disease, and anemia [3]. The methods most frequently used to measure plasma creatinine is based on the Jaffe's reaction first described in 1886 [4]. The objective of this study was to compare between results of plasma creatinine using endpoint Jaffe reaction and Kinetic method in patients with chronic renal failure.

2.0 Materials and Methods:

This study was a case control study (Comparative analytical study). and the study was carried out during October 2011 to March 2012.

Study population

Patients with Renal Failure (plasma creatinine level more than 2 mg/dl), and normal individuals (plasma creatinine level 0.2-1.2mg/dl).

Sampling

This was a hospital based study wherein any renal failure patient admitted to the hospital during the period of the study was enrolled for this study. A total sixty plasma specimens were included in this study (30 samples from healthy individuals and 30 from patients with Renal Failure). After obtaining informed consent and use of antiseptic alcohol swab for skin (70% ethanol), 3 ml of venous blood was collected from each volunteer in this study using a disposable plastic syringe from the antecubital vein or the back of the hand. The blood was then poured in a lithium heparin anticoagulant container and centrifuged at 3000 rpm for 10 minutes to obtain the plasma.

Statistical analysis:

Data was analyzed using Statistical Package for Social Sciences (SPSS).

Quality control:

Normal and pathological commercial control sera were analyzed within each batch.

Ethical consideration:

Permission for this study was obtained from legal authorities in the area of the study. The objectives of the study were explained to all individuals participating in this study. An informed consent was taken from both patients and control groups.

Biochemical measurements:

Plasma creatinine level. Jenway colorimeter

Jaffe reaction:-

Principle:-

Creatinine reacts with picric acid in alkaline solution to form a colored complex.



In the Endpoint Jaffe reaction, the yellow-red complex will be obtained after deproteinization of plasma, but in the Kinetic Jaffe reaction, the final colour will be obtained in a short time (seconds).

Kinetic Jaffe reaction**Reagents**

Reagent -1	creatinine standard	2.0 mg/dl
Reagent-2	picric acid	38 mmol/l
Reagent-3	sodium hydroxide	0.4 mol/l

Linearity

Data suggests that the linearity was found to be 20 mg/dl [1.77 mmol/l].

Working solution [R2+R3]

According to the requirements, working solution was prepared by mixing equal volumes of R2 and R3. The working solution is stable for 6 hours at 20-25 °C, when stored in a dark bottle.

Procedure

Manual procedure:

Wavelength	480-520nm
Cuvette	1cm light path
Temperature	20-25 °C
Zero adjustment	against air or H ₂ O
Specimen	serum, plasma or urine

The following was pipetted in test tube or cuvette:

Working solution	1000µl
Standard or specimen	100 µl

The mixture was mixed and the initial absorbance was read after 30 sec. [A1]. After exactly 2 min. the absorbance was read again [A2].

Calculation

The absorbance of standard and specimen was calculated using the following formulae:

$$\text{Absorbance} = [A2 - A1]$$

Then creatinine concentration was calculated using the following formulae:

$$\frac{\text{Absorbance of specimen}}{\text{Absorbance of standard}} \times \text{standard value}$$

End point Jaffe reaction:**Reagents**

Reagent (1)	picric acid	8.5 mmol/l
Reagent (2)	borate buffer	0.29 mol/l
Creatinine CAL	creatinine standard	2 mg/dl

All the reagents were ready to use.

Procedure

The procedure adapted for estimation of creatinine is as follows

Wavelength	500nm
Cuvette	1cm light path

Temperature 15-25 °c
 Zero adjustment with distilled water
 Specimen serum, plasma or urine

The following was pipetted into test tube

	Blank	Standard	sample
R1 (ml)	-	4.5	4.5
Standard (ml)	-	0.5	-
Sample (ml)	-	-	0.5

Each tube was mixed over a vortex mixer for 15 sec and centrifuged at 2500 rpm for 10 min. Then the following was pipette

	Blank	Standard	Sample
R1 (ml)	2.5	-	-
Supernatant (ml)	-	2.5	2.5
R (2) (ml)	1.0	1.0	1.0

Mixture was mixed and incubated for 20 min. at room temperature. The absorbance (A) of the sample and calibrator was read against the blank.

Calculations

Creatinine value was calculated by using following equation

$$\frac{\text{Absorbance of specimen}}{\text{Absorbance of standard}} \times \text{standard value}$$

3.0 Results and discussion

The results of our study showed a significant p value in the mean and standard deviation of plasma creatinine by end point Jaffe and kinetic Jaffe reaction of the test group and the control group. [Table-1, table-2, figure-2].

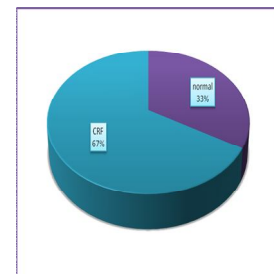
Table-1 Data showing the difference in mean, standard deviation and the P value of plasma creatinine in CRF

Table-1: Data depicting statistical values of employed methods

	N	Mean	Std. Deviation	P.value
kinetic Jaffe	50	3.724	1.4285	0.000*
End point Jaffe	50	3.52	1.417	

The difference in the mean and standard deviation resulted from the difference in time in which the endpoint Jaffe reaction takes longer time due to the multiple procedure steps which allow the interaction of interfering substances and increases the percentage of error and occurrence of false results.

Figure-1: Data showing the percentage of patients to the control.



In kinetic Jaffe reaction there is no time problem because the reaction read immediately after 30 seconds, one minute and two minutes to overcome the effect of interfering substances and there is no time consuming and less procedural steps which minimize the error.

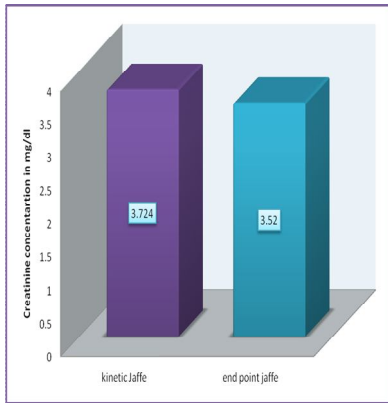


Figure-2: Data showing the difference between the means of kinetic Jaffe reaction and end point Jaffe reaction for patients group.

	N	Mean	Std. Deviation	P.value
kinetic Jaffe	25	0.68	0.1581	0.000*
end point Jaffe	25	0.552	0.1447	

Table-2: Data showing the difference in mean, standard deviation, standard error mean and the P value of the results of plasma creatinine level using kinetic jaffe reaction and end point jaffe reaction for control group.

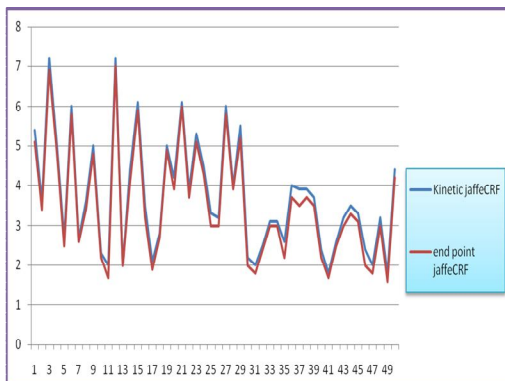
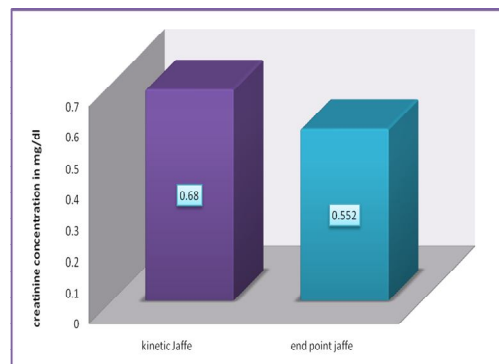


Figure-3: Data showing the difference between the results of plasma creatinine level using kinetic jaffe reaction and end point jaffe reaction for patients group.

Figure-4: Graph depicting the difference between the means of kinetic Jaffe reaction and end point Jaffe reaction for control group.



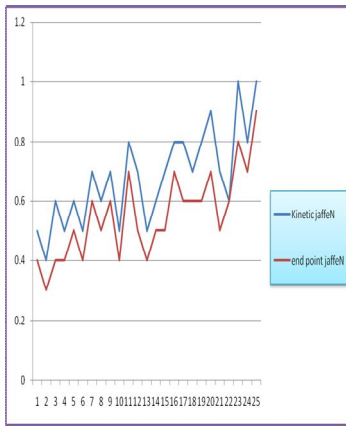


Figure-5: Graph showing the difference between the results of plasma creatinine level using kinetic jaffe reaction and end point jaffe reaction for control group.

The economical aspect of kinetic Jaffe reaction is cheaper than the endpoint Jaffe reaction. Finally our research shows that kinetic Jaffe reaction is better than endpoint Jaffe reaction in accuracy of results, time and cost, and agrees with Folin and Wu H, (1919) [5], who found that the endpoint Jaffe reaction is non specific and subjected to interference by a large number of compounds, and agree with Haeckel R [6,7], who found that the endpoint Jaffe reaction is time consuming and not readily automated. Finally it agrees with Wong ET [8], who found that the kinetic Jaffe reaction is rapid, inexpensive, and easy to perform.

4.0 Conclusion

It is obvious from these results that, kinetic Jaffe reaction is better than endpoint Jaffe reaction as endpoint Jaffe method is time consuming, more affected by interference and expensive than kinetic Jaffe reaction which is less affected by interference and not time consuming.

References:

1. Bishop ML, Janet L, Duben-Engelkirk, Fody EP. Clinical Chemistry, principles, procedures, correlations 5th ed. Lippincott Williams & Wilkins. London 2005; 224-532.
2. Tietz N. fundamental of clinical chemistry. 6th ed. Philadelphia; WB saunders, 2008; 636-647.
3. Marshall WJ. Clinical chemistry 6th ed. London: J.B Lippincott 2008: 75.
4. Jaffe M, Umber den niederschlag welchen pikrinsaure in normalen harn erzeugt und uber eine neue reaction des kreatinins. Z physiol Chem. 1886;10:391-397.
5. Folin, Wu H. System of blood analysis . J Biol Chem. 1919;32:81-86.
6. Haeckel R. Assay of creatinine in serum with use of Fuller's earth to remove interferents. Clin Chem. 1981;27:179-184.
7. Larse K. Creatinine assay by a reaction kinetic principle. Clin Chem Acta. 1972;41:209-217.
8. Wong ET, Bowers LD. Kinetic serum creatinine assays. II. A critical evaluation and review. Clin Chem. 1980;26(5):555-561.