بسم الله الرحمن الرحيم

Sudan University of Science and Technology

Collage of Medical Laboratory Science

Clinical Chemistry Department

Comparison between Kinetic and Endpoint Jaffe Method for Estimation of Creatinine

مقارنة بين طريقتي جافي المستمرة و المنتهية لقياس الكرياتينين في بلازما الدم

A dissertation submitted in partial fulfillment for the requirements of BSc (honor)degree in medical laboratory Science (Clinical Chemistry)

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MSc.(Clinical Chemistry)

July,2014

الآية

قــال تعــالى : (رَبِّ أوْزِعْنِــي أنْ أشْـكُرَ نِعْمَتَـكَ الَّتِـي أنْعَمْـتَ عَلَـيَّ وَعَلَــى وَالِـدَيَّ وَأنْ أعْمَـلَ صَـالِحاً تَرْضَـاهُ وَأدْخِلْنِــي بِرَحْمَتِـكَ فِــي عِبَادِكَ الصَّالِحِينَ).

صدق الله العظيم

سورة النمل الآية 19

Dedication

This research paper is lovingly dedicated to:

Our respective parents

Who have been our constant source of inspiration, they have given us the drive and discipline to tackle any task with enthusiasm and determination. Without their love and support this work would not have been made possible.

Our sisters

Our brothers

And our friends

Who help us in this work

Nosiba and Islam

Acknowledgments

Thank and praise to Allah, who gave us, health, strength and patience to complete this study.

We thank too much our supervisor **Ustaz: Bader Aldeen Musa Ahmed** for his patience, guidance, continuous follow up through this study, God bless him.

Thank also extend to the staff of Clinical Chemistry lab, and students of Sudan University of science and technology in western and southern sections.

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Abstract

This study conducted during the period of February to July 2014, and aimed to compare between endpoint and kinetic Jaffe's method which are commonly used for measurement of creatinine in practice in Sudan.

This study includes 40 samples collected from 40 volunteers (healthy) were selected randomly from the western and southern sections of Sudan University of Science and Technology.

All samples collected in heparin containers and the plasma was separated. Aliquot of plasma was utilized in duplicate to estimate the plasma creatinine level by endpoint method and kinetic method.

All results were analyzed using SPSS program.

This study showed that there was statistical significant difference between creatinine results obtained by using endpoint method (mean = 1.1±.11 mg/dl) and kinetic method (mean = .72±.27 mg/dl) with P.Value = 0.00

This study also reveals that there was statistically significantly increases in creatinine level in males (n=30) (mean=1.1±.11mg/dl) when compared with that of

females (n=10) (mean=1±.11) with P.Value =0.04 in endpoint method.

This study also shows that there was statistically non significantly increases in creatinine level in males (mean=0.74±0.28mg/dl) (n=30) when compared with that of females (n=10) (mean=0.67±0.22mg/dl) with P.Value=0.48 in kinetic method.

From the results of this study, we conclude that There was significant different between the result obtained by kinetic and endpoint methods for creatinine estimation in the plasma.

ملخص البحث

اجريت هذه الدراسة في الفترة من شهر فبراير الى شهر يوليو 2014 للمقارنة بين طريقتي جافى المنتهية والمستمرة لقياس مستوى الكرياتينين في بلازما الدم ، و هي الطرق الاكثر شيوعا في السودان .

تم جمع 40 عينة من المتطوعين الاصحاء من القسمين الجنوبي و الغربي بجامعة السودان للعلوم و التكنولوجيا .

جمعت كل العينات في حاويات الهيبارين ، و تم فصل البلازما من الدم ،و قيس مستوي الكرياتنين في البلازما باستخدام كلا الطريقتين .

النتائج المتحصل عليها تم تحليلها باستخدام برنامج الحزمة الاحصائية للعلوم الاجتماعية .

هذه الدراسة وضحت انه يوجد فروقات ذات دلالة احصائية في مستوي الكرياتينين المتحصل عليه بطريقة جافى المنتهية (الوسط الحسابي =0.11±1.1) ملي جرام لكل ديسيلتر عند مقارنتها بطريقة جافى المستمرة (الوسط الحسابي=0.27±0.72)مليجرام لكل ديسيلتر مع(P.Value=0.00).

اظهرت هذه الدراسة ايضا ان هنالك زيادة ذات دلالة احصائية في مستوى الكرياتينين في الذكور (ن=30) (الوسط الحسابي=0.11±1.1)مليجرام لكل ديسيلتر مقارنة بالإناث (ن=10) (الوسط الحسابي =0.11±1)مليجرام لكل ديسيلتر مع (P.Value=0.04) في طريقة جافى المنتهية.

واظهرت ايضا انه يوجد فرق ذو دلالة غير احصائية في مستوى الكرياتينين في الذكور (ن=30) (الوسط الحسابي=0.28±0.74) مليجرام لكل ديسيلتر

مقارنـــا بالإنـــاث (ن=10) (الوســـط الحســـابي=0.67±0.22)مليجـــرام لكـــل ديسيلتر مع (P.Value=0.48) في طريق جافى المستمرة .

مـن نتـائج هـذه الدراسـة ،فأننـا نسـتنتج ان هنالـك فـرق ذو دلالـة احصـائية بـين النتـائج المتحصـل عليهـا بطريقتـي جـافى المنتهيـة والمسـتمرة اللتـان تسـتخدمان لتقدير مستوي الكرياتينين في بلازما الدم .

CHAPTER ONE

Introduction and Literature Review

1. Introduction and literature review

1.1. Creatinine:

1.1.1. Definition:

Creatinine is formed from creatine and creatine phosphate in muscle and is excreted into the plasma at constant rate related to muscle mass. Plasma creatinine is inversely related to glomerular filtration rate (GFR) and, although an imperfect measure, it is commonly use to assess renal filtration function. [1]

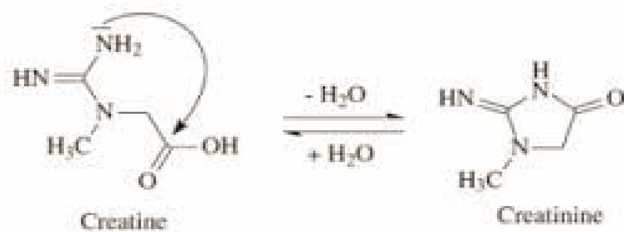


Figure (1.1): Conversion of creatine to creatinine.

1.1.2. Biochemistry and physiology:

Creatinine is synthesized in the (1) kidneys, (2) liver, and (3) pancreas by two enzymatically mediated reactions. In the first, transamidation of arginine and lysine forms guanidinoacetic acid. In the second reaction, methylation of guanidinoacetic acid occurs with S-adenosylmethionine as the methyl donor. Creatine is then transported in blood to other organs, such as muscle and brain, where it is phosphorylated to phosphocreatine, a high-energy compound. [2]

Interconversion of phosphocreatine and creatine is particular feature of metabolic processes of muscle contraction. A proportion of the free creatine in muscle (thought to be between 1%and 2%\day) spontaneously and irreversibly converted to it is and hydride waste product-creatinine. Thus the mount of creatinine produced each day is relatively constant and is related

to the muscle mass. In health, the concentration of creatinine in the blood stream also is relatively constant. However, depending on the individual´s meat intake, diet may influence the value. Creatinine is present in all body fluids and secretions, and is freely filtered by the glomerulus. Although it is not reabsorbed to any great extent by the renal tubules, there is a small but significant tubular secretion. Creatinine production also decreases as the circulating level of creatinine increases; several mechanisms for this have been proposed, including (1) feedback inhibition of production of creatine, (2) reconversion of creatinine to creatine, and (3) conversion to other metabolites.[2]

1.1.3. Serum creatinine:

Measuring serum creatinine is a simple test, and it is the most commonly used indicator of renal function. [3]

A rise in blood creatinine level is observed only with marked damage to functioning nephrons. Therefore, this test is unsuitable for detecting early-stage kidney disease. A better estimation of kidney function is given by calculating the estimated glomerular filtration rate (eGFR). eGFR can be accurately calculated using serum creatinine concentration and some or all of the following variables: sex, age, weight, and race, as suggested by the American Diabetes Association without a 24-hour urine collection.[4]

1.1.4. Plasma creatinine (PCr):

Plasma creatinine concentration is the most reliable simple biochemical test of glomerular function. Ingestion of stewed meat can increase plasma creatinine concentration by as much as 30% seven hours after a meal an ideally blood samples should be

collected after an overnight fast. Strenuous exercise also causes a transient, slight increase in plasma creatine concentration. Plasma creatine concentration is related to muscle bulk and therefore a concentration of 120um/l could be normal for an athletic young man but would suggest renal impairment, though not necessarily of clinical significance, in a thin, 70-year-old women. Although muscle bulk tends to decline with age, so too does the GFR and hence plasma creatinine concentration remain fairly constant. (5)

Normal value of plasma creatinine:

• For adult male: 0.9 - 1.3 mg/dl.

• For adult female: 0.6 - 1.1 mg/dl. (2)

Low plasma creatinine:

A low [creatinine] is found in subjects with a small total muscle mass. Low plasma [creatinine] may therefore be found in children, and value is, on average, normally lower in women than in men. Abnormally low values may be found in wasting diseases and starvation, and in patients treated with corticosteroids, due to their protein catabolic effect. creatinine synthesis in is increased in pregnancy, but this is more than offset by the combined effects of the retention of fluid and the physiological rise in GFR that occur in pregnancy, so plasma [creatinine] is usually low.

High plasma creatinine:

Plasma [creatinine] tends to be higher in subjects with a large muscle mass. other non-renal causes of increase plasma [creatinine] include the following:

- A high meat intake can cause temporary increase.
- Transient, small increases may occur after vigorous exercise.

- Some analytical methods are not specific for creatinine.
- Some drugs (e.g.: salicylates, cimetidine) complete with creatinine for its tubular transport mechanism, thereby reducing tubular secretion of creatinine and elevating plasma [creatinine].
- Impaired renal perfusion (e.g.: reduced blood pressure, fluid depletion, renal artery stenosis).
- Loss of functioning nephrons (e.g.: acute and chronic glomerular nephritis).
- Increase in the tubular side of the nephron (e.g.: urinary tract obstruction due to prostatic enlargement).[6]

1.1.5. Creatinine and creatinine clearance:

Creatinine is a nearly ideal substance for the measurement of clearance for various reasons: it is an endogenous metabolic product synthesized at a constant rate for a given individual, it is cleared essentially only by glomerular filtration, and it can be analyzed in expensively by readily available colorimetric methods. As the result, creatinine clearance has become the standard laboratory assay for determination of early renal failure. This value is derived by arithmetically relating the total urine creatinine concentration to total serum creatinine concentration excreted during 24-hour period. [7]

Specimen collection, therefore, must include both 24-hour urine specimen and a serum creatinine value (ideally taken at the midpoint of the 24-hour urine collection and, realistically, no letter than 24-hours before or after the urine collection). The urine container (clean, dry, and free of contaminants or preservatives) must be kept refrigerated throughout or the duration of both the collection procedure and the subsequent storage period until

laboratory analyses can be performed. The patient must maintain an adequate fluid intake throughout the procedure to insure a minimum urines flow rate of at least 2ml per minute. This cancels out the possibility of negative error due to urine retention in the bladder. [7]

Once the specimens have been collected, the concentration of creatinine in both serum and urine is measured by any of the applicable method .the total volume of urine is carefully measured.

Calculation:

The creatinine clearance is calculated by the following formula:

$$C_{cr}\ (\text{ml/min}) = \frac{U\ cr\ (\text{mg/dl}) \times Vur\ (\text{ml/24h})}{Pcr \times 24h} \times \frac{1.73}{A}$$

Whereas C_{cr} =creatinine clearance.

U_{cr} = urine creatinine concentration

V_{ur}=urine volume execrated in 24 hours

P_{cr}= serum creatinine concentration

1.73/A = normalization factor for body surface area

Normally, the urine output of healthy individual averages 1500ml/24h; because this total volume is only about 1% of the glomerular filtrate formed each day, a large value is reabsorbed elsewhere along the nephron. [7]

1.1.6. The significance of creatinine:

Creatinine is produced endogenously and released into body fluid at constant rate and its plasma concentration is maintained within narrow limits predominantly by glomerular filtration.

Consequently, both plasma creatinine concentration and its renal clearance (creatinine clearance) have been used as markers of the glomerular filtration rate (GFR). [2]

1.2. Renal failure:

1.2.1. Definition:

is a medical condition in which the kidneys fail to adequately filter waste products from the blood.[8]The two main forms are acute kidney injury, which is often reversible with adequate treatment, and chronic kidney disease, which is often not reversible.[9]

1.2.2. Acute kidney Injury:

Acute kidney injury is a sudden, sharp decline in renal function as a result of an acute toxic or hypoxic insult to the kidneys. AKI is a common and serious condition that occurs in approximately 5% of all hospitalized patients. The risk, injury, failure, loss of function, end-stage renal disease (RIFLE) and the acute kidney injury network definitions of AKI are based on changes in both serum creatinine and urinary output; however, both display poor specificity and sensitivity for the early detection of AKI.[10]

Novel urine and plasma biomarkers, such as NGAL and kidney injury molecule-1 (KIM-1), are emerging as excellent biomarkers for the early prediction and prognosis of AKI. AKI is subdivided into three types, depending on the location of the precipitating defect.[10]

Prerenal AKI: the defect lies in the blood supply before it reaches the kidney. Causes can include cardiovascular system failure and consequent hypovolemia.[10]

Intrinsic AKI: the defect involves the kidney. The most common causes are acute tubular necrosis; other causes include vascular obstructions/ inflammations and glomerulonephritis. [10]

Postrenal AKI: The defect lies in the urinary tract after it exits the kidney. Generally acute renal failure occurs as consequences of lower urinary tract obstruction or rupture of the urinary bladder.[10]

Toxic insult to the kidney that are severe enough to initiate acute renal failure include hemolytic transfusion reactions, myoglobinuria due to rhabdomyolysis, heavy metal poisoning, antifreeze ingestion, and analgesic and amino glycoside toxicities. These conditions directly damage the renal tubules. Hypoxic insults include conditions that severely compromise renal blood flow, such as septic/hemorrhagic shock, burns, and cardiac failure. The most commonly observed symptoms of acute renal failure are oliguria and anuria (<400ml/d). The diminished ability to excrete electrolyte and water result in a significant increase in extracellular fluid volume, leading to peripheral edema, hypertension, and congestive heart failure. Most prominent, however, is the onset of the uremic syndrome or kidney failure, in which increase BUN and serum creatinine values are observed along with the preceding symptoms the outcome of this disease is either recovery or in the case of irreversible renal damage progression to chronic renal failure.[10]

1.2.3. Chronic kidney disease:

Chronic kidney disease is a clinical syndrome that occurs when there is a gradual decline in renal function over time. Early detection and treatment are needed to prevent progression to kidney failure and complication such as coronary vascular disease. GFR an evidence of kidney damage based on measurement of proteinuria or others markers form the bases of classification. The conditions that can precipitate AKI also may lead to chronic renal failure. [10]

1.2.4. Acute on chronic renal failure:

Acute renal failure can occur and precipitate presentation in patients with pre-existing (chronic) renal impairment. In practice often necessary to manage the acute event and investigate the patient for chronic renal disease latter.[11]

Factors suggesting and element of chronicity in acute renal failure (acute on chronic renal failure): Absence of acute illness, Long duration of symptoms, Nocturia, Anaemia, Bone disease, Sexual dysfunction, Neuropathy, Skin disorders, and Small kidneys demonstrated by imaging. [11]

1.3. Measurement of creatinine:

1.3.1. Chemical method:

Jaffe´s reaction:

In this method, creatinine reacts (nonspecifically) with picric acid in alkaline media to give red color chromogen (creatinine and psuedocreatinine –picrate complex) which can be measure at 490nm (blue to green filter).

A serious analytical problem with the Jaffe´s reaction is its lack of specificity for creatinine. For example, many compound have

been reported to produce a Jaffe-like chromogen, including (1)ascorbic acid. (2)blood-substitute products, (3)cephalosporin, (4)glucose ,(5)guanidine, (6)ketone bodies, (7)protein ,and (8)private. The degree of interference from these compounds is dependent on the specific reaction conditions chosen. [2]

(a) Jaffe´s kinetic procedure:

In this procedure creatinine is measured within one method after 30 second from addition of sample to alkaline picrate, since creatinine was thought to be reacting with alkaline picrate within one minute after 30 second fro addition of sample to alkaline picrate. Within 30 second, acetoacetate was thought to be reacting if present in serum or plasma.

Advantage:

Kinetic assays have been developed to provide more specific, faster, and automated analyses. [2]

Disadvantage of this method:

Pseudo-creatinine was represented if time is not adjusted accurately.

(b) Jaffe´s endpoint procedure:

In this procedure Pseudo-creatinine were minimized by adsorbing to precipitate during deprotienization step.

Advantage of this method:

easy to prepared in the lab, linearity is up to 5mg/dl, cheap, adapted for use on auto-analyzer, and Pseudo-creatinine were thought to be adsorbed to precipitate whereas true creatinine was adsorbed to supernatant or filtrate .

Disadvantage of this method:

This include deprotienization step with many hazard factors, not sensitive, and not specific. [12]

1.3.2. Enzymatic methods:

Enzymes for a number of metabolic pathways have been investigated for the enzymatic measurement of creatinine. All of the method involved a multistep approach leading to photometric equilibrium. There are primary three approaches, describe belong.

Creatininase:

Creatininase (E.C.3.5.2.10; creatinine iminohydrolase) catalyzes the conversion of creatinine to creatine. the creatine then detected with a series of enzyme-mediated reaction involving creatine kinase, private kinase, and lactate dehydrogenate, with monitoring of the decrease in absorbance 340nm. Initiating the reaction without creatininase allows for the removal of endogenous creatine and private in pre-incubation reaction. the kinetics of the reaction are analytically problematic and a 30-minute incubation is the required to allow the reaction to reach equilibrium .This shortcoming has been overcome by a kinetic approach but a further reduction in the method's ability to detected creatinine consequently, this is approach is not widely use .

Creatinine delaminate:

Creatinine delaminate (EC 3.5.4.21; creatinine iminohydrolase) catalyzes conversion of creatinine to N-methylhydantion and ammonia. Early methods concentrated on detection of ammonia using either glutamate dehydrogenate or the Berthelot reaction. An alternative approach involves the enzyme N-methylhydantion amiiminohydrolase.

Creatininase and Creatinase:

An alternative approach has been use of creatinase (E.C.3.5.3.3; creatine amidinohydrolyase that yields sarcosine and

urea, the formal being measured is further enzyme-mediated steps using sarcosine oxidase (EC.1.5.3.1). this produces (1)lysine (2) formaldehyde, and (3) hydrogen peroxide with the latter being detected and measured with a variety of methods. care must be taken , however, because of interference (e.g. , bilirubin) in the final reaction sequence. this problem has been minimized by adding potassium ferricyanide or bilirubin oxidase. the potential interference caused by ascorbic acid has been overcome by inculcation of ascorbate oxidase (L-ascorbate: oxygen oxidoreductase ; EC.1.10.3.3). the influence of endogenous intermediate creatine and urea has been minimized by adding a preincubation step and then initiating the reaction with creatininase. This system has been incorporated in a point of care testing device using polar graphic detection. And alternative detection system involves measurement of the reduction of nicotinamide adeninedinuclotide (NAD) by formaldehyde in the presence of formaldehyde dehydrogenate. [2]

1.4. Objectives:

1.4.1. General objective:

- To compare between kinetic and end point Jaffe method for creatinine measurement.

1.4.2. Specific objectives:

- To determine creatinine in study groups by kinetic Jaffe method.
- To determine creatinine in study groups by end point Jaffe method.
- To compare between the two above methods in term of results, time Consume feasibility to perform, availability and cost.

CHAPTER TWO

Materials and Methods

2. Materials and method

2.1. Materials:

2.1.1. Study design:

This is cross-sectional, analytical, comparative study

2.1.2. Study area:

The study was conducted in western and southern sections of Sudan University of science and technology.

2.1.3. Study population:

this study include 40 healthy people, students in western and southern departments of Sudan university of science and technology, age between 18-25 years old, 10 females and 30 males.

2.1.4. Sampling:

Venous blood sample (4ml) were collected by standard Procedure from each participates in containers containing heparin and centrifuged for 3 minutes at 3000 rpm. The clear plasma which obtained was stored at -20c, until used for analysis.

2.1.5. Reagents:

1) Reagent used for kinetic:

Sodium hydroxide + picric acid (equal volume)

2) Reagent used end point method:

These reagents were prepared in laboratory and include:

R1 which contains: 5%Sodium tungstate.

R2 which contains: 2/3 N. Sulphuric acid.

R3 which contains: 0.75%N of NaOH.

R4 which contains: Picric acid.

2.1.6. Equipment:

- Colorimeter.

- Centrifuge.

2.2. Methodology:

Method used for estimation of creatinine are kinetic method and End point method, were used in this study.

2.2.1. Kinetic method:

Principle:

Creatinine in sample reacts with alkaline medium forming a colored Complex .The complex formation rate is measured in a short period to avoid interference. The rate of formation of color is proportional to the creatinine Concentration in the sample.[12]

Procedure for Kinetic method:

The reagents were prepared and filter was adjusted at 490nm,and then zero was adjusted with the empty cuvette against blank.0.1ml of sample was added to 1.0 ml of working reagent in test tube, and then the tube was mixed and immediately transferred to cuvette. After exactly thirty (30) seconds the absorbance (A1) was recorded. After exactly sixty (60) seconds after A1 reading again the absorbance (A2) was recorded. The above were repeated for creatinine estimation for standard.

Calculation:

$\Delta AT = A2 - A1 \qquad \Delta ASTD = A2 - A1$

Concentration of T = $\frac{\Delta Abssample}{\Delta Absstandard}$×concentration of STD =mg/dl

2.2.2. End point method:

Principle:

Creatinine reacts with picric acid in alkaline medium to give yellow to red colored complex which absorbed light at 490nm.

Procedure:

Two steps:

1-Deproteinization step:

The dry centrifuge tube was prepared as follow:

0.5ml (plasma) +0.5ml (D.W) +0.5ml (R1) +0.5ml (R2)

The tube was mixed and let stand for 5 min, then was centrifuged

At 3000 rpm for 5 min. then continued as follow:

There test tube was prepared as follow:

	Blank	Standard	Sample
Standard(0.5mg/dl)	_	0.9ml	_
Supernatant	_	_	0.9ml
D.W	0.9mL	_	_
R3	0.3	0.3	0.3ml
R4	0.3	0.3	0.3ml

The tubes were mixed and incubated for 15 min at R.T, and then theabsorbancewas read against blank reagent at 490 nm.

Calculation:

Concentration of T = $\frac{\Delta Abssample}{\Delta Absstandard}$×concentration of STD ×D.F (4)

2.3. Data analysis:

The data was recorded and analyzed using statistical package for social sciences (SPSS version 11.5) on programmed computer.

2.4. Ethical considerations:

All participants were told about the research importance during interview and all of them were agreed to participate.

2.5. Quality control:

It recommended to use the biochemistry control serum level I (cod.18005, 18009, and18042) and II (cod. 18007, 18010, and

18043) and the biochemistry control urine (cod.18054) to verify the performance of the measurement procedure.
Each laboratory should establish its own Internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

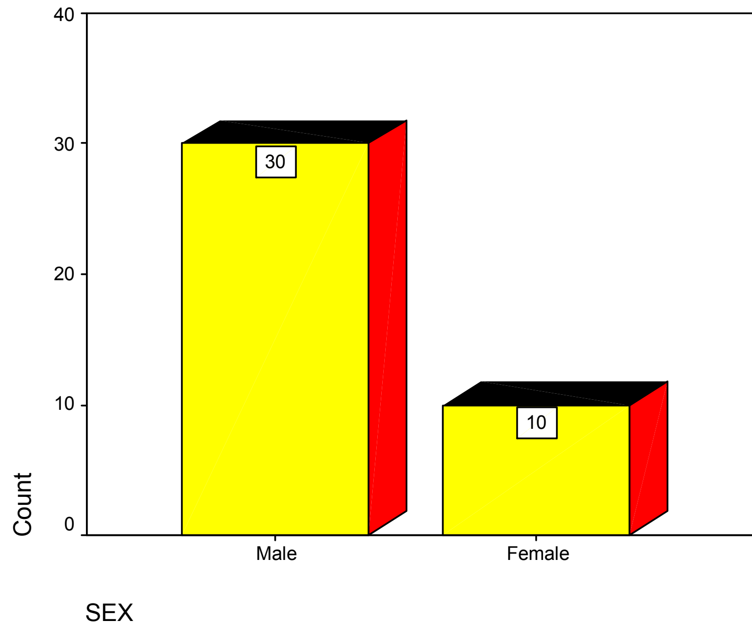
CHAPTER THREE

Results

3. Results

The table (3.1) shows descriptive statistics of date.

Method	N		Minimum	maximum	Mean	Std. Deviation
	Male	Female				
Kinetic mg/dl	30	10	0.6	1.3	0.72	0.27
Endpoint mg/dl	30	10	1.0	1.3	1.12	0.11

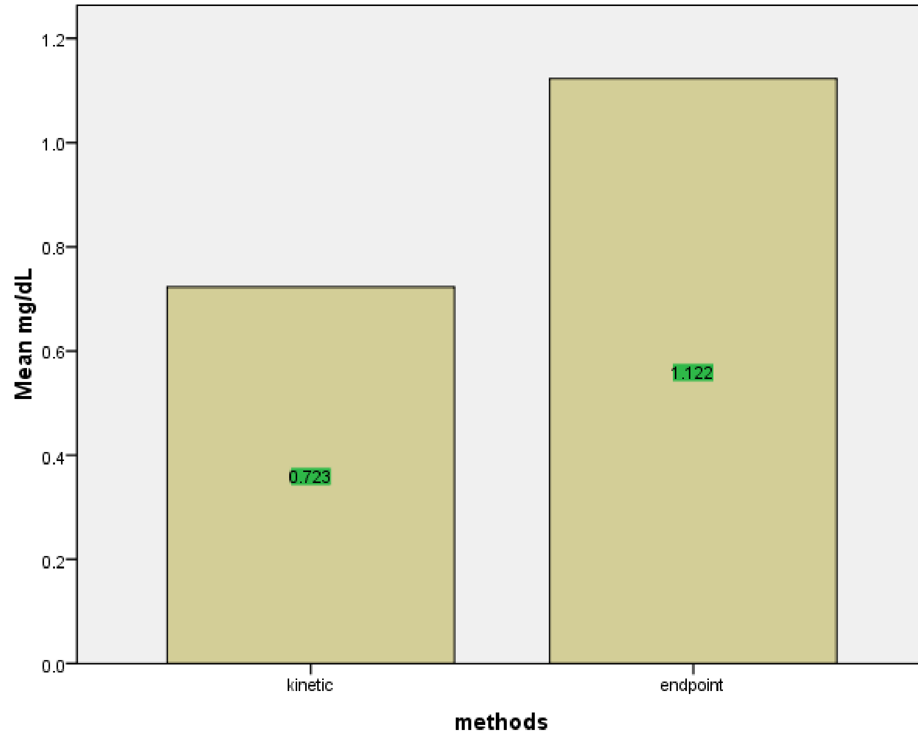


The figure (3.1) shows distribution of individual according to Gender.

The table (3.2) Shows the mean and standard deviation , and level of significance (P. Value). Independent T-test was used for comparison between the test groups.

P. Value ≤ 0.05 was considered significant.

Methods	N	Mean	Std. Deviation	P .Value
Kinetic	40	.723	.2694	.000
Endpoint	40	1.122	.1143	



The figure (3.2) shows mean of results obtained by two methods .

The table(3.3) Shows the mean, standard deviation and level of significance according to the sex .

P.Value ≤0.05 was considered significant.

Methods	Sex	N	Mean	Std. Deviation	P .value
Kinetic method Mg/dl	Male	30	0.74	0.2848	0.484
	Female	10	0.67	0.2214	
Endpoint method mg/dl	Male	30	1.143	0.1104	0.044
	Female	10	1.06	0.1075	

CHAPTER FOUR

Discussion, Conclusions and Recommendations

4 Discussion, conclusions and recommendations

4.1 Discussion:

This is an analytical comparative study aimed to compare between kinetic and endpoint Jaffa methods for estimation of creatinine .

This study are included 40 populations selected randomly (healthy people), 30 persons of them are male while the other are female.

Heparinized blood samples were collected from study population and estimated for creatinine by the above method (kinetic and Endpoint). The results were analyzed using independent T test of computerized SPSS program.

As indicated in table (3.2) there was statistically significant different between the result obtain by kinetic method (mean = .72±.27 mg/dl) and result obtained by endpoint method (mean = 1.1±.11 mg/dl) with (P.Value 0.00) during the study group.

Also in table (3.3), the results obtained by endpoint statistically significantly higher in male (mean=1.1±.11mg/dl) than female (mean=1±.11) with P.Value =0.04, and the results obtained by kinetic method were statistically non-significant increase in males (mean=0.74±.28mg/dl) than females (mean=0.67±.22mg/dl) with P.Value=0.48.

This result was agreed and compatible with study (Plasma creatinine results derived from an endpoint modification of the

Jaffé method) by Scott J. Schurman, Sharon A. Perlman, Wilfredo Chamizo which revealed that the endpoint measured plasma creatinine higher by about 0.1 -0.3 mg/dl according to age than kinetic method with ($P.Value<0.0001$) . The combination of these higher values and the same reported normal range for all children ages 2–12 years (0.3–1.0 mg/dl) and 13–17 years (0.7–1.4 mg/dl) makes interpretation of Olympus AU5000 endpoint method results difficult, particularly for younger children. [13]

4.2. Conclusions:

- There was significant different between the result obtained by kinetic and endpoint methods for creatinine estimation.
- Kinetic method gives results significantly higher in males than females, and endpoint gives results insignificantly higher in males than females.
- Endpoint method gives results higher than kinetic method.

4.3. Recommendations:

- Further studies to see the most suitable method for creatinine estimation by using reference methods with large sample size.
- The results reinforce the need for each laboratory to provide comprehensive age- and sex-adjusted normal Plasma creatinine ranges.

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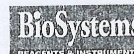
Appendix

COD 11802 2 x 50 mL	COD 11502 4 x 50 mL	COD 11542 1 x 1 L
STORE AT 15-30ºC		
Reagents for measurement of creatinine concentration Only for *in vitro* use in the clinical laboratory		

CREATININE



CREATININE
ALKALINE PICRATE

PRINCIPLE OF THE METHOD

Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex. The complex formation rate is measured in a short period to avoid interferences[1,2].

CONTENTS

	COD 11802	COD 11502	COD 11542
A. Reagent	1 x 50 mL	2 x 50 mL	1 x 500 mL
B. Reagent	1 x 50 mL	2 x 50 mL	1 x 500 mL
S. Standard	1 x 5 mL	1 x 5 mL	1 x 5 mL

COMPOSITION

A. Reagent. Sodium hydroxide 0.4 mol/L, detergent.

Irritant (Xi): R36/38: Irritating to eyes and skin. S26: In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. S37/39: Wear suitable gloves and eye/face protection.

B. Reagent. Picric acid 25 mmol/L.

S. Glucose/Urea/Creatinine Standard. Glucose 100 mg/dL, urea 50 mg/dL, creatinine 2 mg/dL (177 µmol/L). Aqueous primary standard.

STORAGE

Store at 15-30ºC.

Reagents and Standard are stable until the expiry date shown on the label when stored tightly closed and if contaminations are prevented during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, absorbance of the blank over 0.350 at 500 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

REAGENT PREPARATION

Standard (S) is provided ready to use.

Working Reagent: Mix equal volumes of Reagent A and Reagent B. Mix thoroughly. Stable for 1 month at 2-8ºC.

ADDITIONAL EQUIPMENT

- Thermostatic water bath at 37ºC
- Analyzer, spectrophotometer or photometer able to read at 500 ± 20 nm.

SAMPLES

Serum, plasma or urine collected by standard procedures. Dilute fresh urine 1/50 with distilled water before measurement. Heparin, EDTA, oxalate and fluoride may be used as anticoagulants.

Creatinine in samples is stable for 24 hours at 2-8ºC.

PROCEDURE

1. Bring the Working Reagent and the photometer to 37ºC.
2. Pipette into a cuvette: (Note 1)

Working Reagent Standard (S) or Sample	1.0 mL 0.1 mL

3. Mix and insert cuvette into the photometer. Start stopwatch.
4. Record the absorbance at 500 nm after 30 seconds (A_1) and after 90 seconds (A_2).

CALCULATIONS

The creatinine concentration in the sample is calculated using the following general formula (Note 2):

$$\frac{(A_2-A_1)_{\text{Sample}}}{(A_2-A_1)_{\text{Standard}}} \times C_{\text{Standard}} \times \text{Sample dilution factor} - \text{Corrective Factor}^{4,5} = C_{\text{Sample}}$$

If the Creatinine Standard provided has been used to calibrate (Note 3):

	Serum and plasma	Urine
$\left[\frac{(A_2-A_1)_{\text{Sample}}}{(A_2-A_1)_{\text{Standard}}}\right.$	x 2] -0.37 = mg/dL creatinine	x 100 = mg/dL creatinine
	x 177] -33 = µmol/L creatinine	x 8840 = µmol/L creatinine

REFERENCE VALUES

Serum and plasma[3]:

Men: 0.9-1.3 mg/dL = 80-115 µmol/L
Women: 0.6-1.1 mg/dL = 53-97 µmol/L

Urine[3]:

Men: 14-26 mg/kg/24-h = 124-230 µmol/kg/24-h
Women: 11-20 mg/kg/24-h = 97-177 µmol/kg/24-h

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007, 18010 and 18043) and the Biochemistry Control Urine (cod. 18054) to verify the performance of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if controls do not recover within the acceptable tolerances.

METROLOGICAL CHARACTERISTICS

- Detection limit: 0.03 mg/dL creatinine = 2.65 µmol/L creatinine
- Linearity limit: 20 mg/dL = 1768 µmol/L creatinine. For higher values dilute sample 1/2 with distilled water and repeat measurement.
- Repeatibility (within run):

Mean concentration	CV	n
1.7 mg/dL = 150 µmol/L	2.9 %	20
5.3 mg/dL = 468 µmol/L	1.3 %	20

- Reproducibility (run to run):

Mean concentration	CV	n
1.7 mg/dL = 150 µmol/L	3.9 %	25
5.3 mg/dL = 468 µmol/L	2.9 %	25

- Sensitivity: 31 mA·dL/mg = 0.351 mA·L/µmol
- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents (Note 2). Details of the comparison experiments are available on request.
- Interferences: Hemoglobin (10 g/L), bilirubin (10 mg/dL), protein and ketonic bodies do not interfere. Lipemia (triglycerides > 2 g/L) may interfere. High concentration of reducing compounds may interfere. Other drugs and substances may interfere[6].

These metrological characteristics have been obtained using an analyzer. Results may vary if a different instrument or a manual procedure are used.

DIAGNOSTIC CHARACTERISTICS

Creatinine is a catabolic end product of creatine (or phosphocreatine). The amount produced each day is related to the muscle mass. Creatinine is freely filtered by the glomerulus (small amounts are reabsorbed and are also secreted by the renal tubules).

Creatinine measurement is used almost exclusively in the assessment of kidney function (impaired renal perfusion, loss of functioning nephrons) and in the monitoring renal dialysis[3,7].

Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

NOTES

1. These reagents may be used in several automatic analysers. Instructions for many of them are available on request.
2. For measurement in serum or plasma, introduce the corrective value for the reaction of nonspecific proteins as a constant factor subtracted from the concentration value obtained[4,5].
3. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).

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