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Research Article

Interpretation Tool for Creatinine Related Parameters Established using Enzymatic Sarcosine Oxidase Quantitative Analytical Method

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Abstract: The purpose of the study was to establish the reference ranges of serum creatinine and estimated creatinine clearance for adult Kenyan population using enzymatic sarcosine oxidase quantitative analytical method**.** A prospective study carried out in clinical chemistry laboratory of Kenyatta National Hospital involving 631 individuals between 18-61 years. Results for 316 study subjects (158 renal patients and 158 healthy subjects) were used to compare the difference between enzymatic sarcosine oxidase and modified jaffes reaction methods. Comparison of the two methods showed statistically significant difference (renal patient: $p = 0.024$, healthy subjects: $p = 0.038$). Parametric methods were used to construct reference ranges by estimating 2.5 and 97.5 percentiles of distribution as lower and upper reference limits. Results for 462 blood donor study subjects comprising of 249 male and 213 females were used for the establishment of reference ranges of:- serum creatinine and estimated creatinine clearance. Serum creatinine and estimated creatinine clearance reference ranges established were as follows:- serum creatinine {male: (41-109) µmol/L, female: (32-92) µmol/L} and estimated creatinine clearance {male: (36-98) ml/min, female: (31-81) ml/min}. The current study has established adult Kenyan reference ranges for serum creatinine and estimated creatinine clearance using enzymatic sarcosine oxidase analytical method. Enzymatic sarcosine oxidase method produced low creatinine concentration than the modified jaffes reaction method for the studied parameters.

Keywords: Adult Kenyan, estimated creatinine clearance, Kenyatta National Hospital, reference range

INTRODUCTION

Reliable creatinine measurements in both serum and plasma which are used in Glomerular Filtration Rate (GFR) estimation are critical to ongoing global public health efforts to increase the diagnosis and treatment of Chronic Kidney Disease (CKD). Understanding by laboratorians worldwide of the importance of reliable serum creatinine measurements in GFR estimation and of factors that may affect creatinine measurement have been widely emphasized (Arogundade and Barsoum, 2008). The methods most widely used to measure serum/plasma and urine creatinine are alkaline picrate methods, enzymatic or partially enzymatic assays and HPLC methods. Isotope-Dilution Mass Spectrometry (IDMS) high-order reference methods have been developed for assignment of reference materials but are available in only a few highly specialized laboratories worldwide. Today, the Jaffe reaction using alkaline picrate remains the cornerstone of most current routine methods, after continuous refinements attempting to overcome inherent analytical interferences and limitations **(**Miller *et al*., 2005). The kinetic method decreases interference caused by Jaffe-reactive pseudo-creatinine of non-creatinine chromogens compared with earlier protocols. Other methods have been used to improve the specificity of the Jaffe reaction, but they are not suitable in automated procedures. Coupled enzymatic reactions have been developed which reduce or eliminate problems with most interfering substances that the cornerstone method has not successively eliminated. Inorganic chemical–based methods that have been developed as alternatives to the alkaline picrate methods have not been widely implemented clinically because they have not demonstrated improved performance compared with the various adaptations of the Jaffe method. The only alternative methods that have been widely adopted for routine clinical laboratory use are enzymatic creatinine methods specifically the sarcosine-oxidase method.

Although the enzymatic methods have been reported to have generally less interference than the

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Jaffe methods, there have been reports of various substances that do interfere (Myers *et al*., 2006).

Cockcroft and Gault and Modification of Diet in Renal Disease (MDRD) formulas have been used to estimate glomerular filtration rate of the kidney. The creatinine value used in either of these equations is determined using the routinely used creatinine determination methods either kinetic jaffes or enzymatic method. Because no systematic differences between serum and plasma measurements have been reported, serum and plasma results are considered as equivalent**,** (Peake and Whiting, 2006). There is now ongoing activity to promote world-wide standardization of methods to measure creatinine concentrations, together with the introduction of a revised estimated glomerular filtration rate equation appropriate for use with standardized creatinine methods (Panteghini, 2008).

The current study was undertaken using modified kinetic Jaffes reaction and enzymatic sarcosine creatinine determination methods. Sarcosine oxidase enzymatic method was evaluated since it is to be used for the first time in clinical chemistry laboratory of Kenyatta National Hospital. The current study was to compare the two creatinine analytical methods and established reference ranges for serum creatinine and estimated creatinine clearance based on enzymatic sarcosine oxidase method. The Kenyatta National Hospital Research and Ethical Committee approved the study (P459/08/2013).

MATERIALS AND METHODS

Study site: The study was conducted in the department of laboratory medicine, clinical chemistry laboratory of Kenyatta National Hospital.

Study period: May-November 2014.

Study Population: Six hundred and thirty one individuals comprising of 328 males and 303 females between the 18-61years were recruited into the study. Four hundred and seventy three were healthy blood donors while the remaining 158 were renal patients. A questioner was administered to consenting study subjects (blood donors) to gather some socio-demographic data.

Specimen analysis: Three milliliters of blood was collected from each study subject. Serum was separated by centrifugation at 3000 g for 3 min. Studied creatinine levels were determined by modified jaffes reaction and enzymatic sarcosine oxidase methods using autoanalyzer Mindray 800 machine. Specific assayed normal and pathological sera were used for the quality control of analytical work during study period. The following formula was used for the determination of estimated creatinine clearance (Ecrcl):

Ecrcl = {(140-age) x weight (kg) x constant (male = 1.23, female = 1.04)} divide by Serum Creatinine (µmol/L)

The weight of each study subject was taken by weighing using a weigh balance.

RESULTS

The male study participants had a mean age and weight of 33 years and 68kgs respectively. On the other hand the female study subjects had a mean age and weight of 31 years and 67 kgs. Internal quality control report for both normal and pathological control sera were within the specific assigned QC range of target value±2 standard deviations as shown in Table 1.

Serum creatinine quantitative results for 316 study subjects (158 renal patients and 158 healthy subjects) were used to compare the difference between enzymatic sarcosine oxidase and modified jaffes reaction methods. Creatinine results for the renal patients using enzymatic sarcosine oxidase and modified jaffes reaction methods had a mean of 285 and 317 µmol/L, respectively. Paired means comparison test for the two methods showed statistically significant differences between the two creatinine quantitative methods ($p<0.024$) as shown in Table 2. Creatinine results for the healthy subjects using enzymatic sarcosine oxidase and modified jaffes reaction methods had a mean of 69µmol/L and 80µmol/L respectively. Paired means comparison test for the two methods showed statistically significant differences between the two creatinine quantitative methods ($p<0.038$) as shown in Table 2.

Paired means comparison test using results of equal number of male (n = 213) and female (n = 213) healthy

Table 1: Internal quality control report of the studied parameter

			Assigned QC report			Study QC report		
Method	Parameter	QC type	Session	Range	Mean	Mean	Sd	CV%
Jaffes reaction	Scr	n	25	81-135	108	105	4.5	4.2
		p	25	132-260	196	200	15	7.5
Sarcosine	Scr	n	67	56-98	77	79	7	8.9
Oxidase		p	25	67-133	100	102	5.1	5

Scr: Serum creatinine; QC: Quality control; N: normal control sera; P: Pathological control sera; SD: Standard deviation; CV: Coeficient of variation

Table 2: Comparison between enzymatic sarcosine oxidase and modified jaffes reaction methods for quantitative analysis of serum creatinine

Study subjects	N	Method	Mean	MD	S.E	95 C.I		*Sig.
Renal patients	158	Enzymatic sarcosine oxidase	285	32	5.9	L	U	0.024
		Modified jaffes reaction	317			20	44	
Healthy subjects	158	Enzymatic sarcosine oxidase	69	10	0.5	10	12	0.038
		Modified jaffes reaction	80					

*The mean difference significance at p<0.05 level; N: Number; MD: Mean difference; S.E: standard error; CI: Confidence interval

Table 3: Established reference ranges for serum creatinine and estimated creatinine clearance for studied Kenyan population using enzymatic sarcosine oxidase method

Parameter	Gender	n	Mean	MD	Mean±1.96SD	Rr	*Sig
Scr (µmol/L)	Male	249	75	13	75±34	41-109	0.014
	Female	213	62		62±30	32-92	
Ecrcl (ml/min)	Male	249	67	11	67±31	36-98	0.018
	Female	213	56		56±25	31-81	

Nb/Scr: Serum creatinine; Ecrcl: Estimated creatinine clearance; n: number; MD: Mean difference; Rr: Reference range; *Mean difference significance at p<0.05 level

Table 4: Comparision of enzymatic sarcosine oxidase and modified jaffes reaction method established reference ranges for creatinine related parameters for the Kenyan population

Parameter	Method	Sex	Number	Rr
Scr	Enzymatic sarcosine oxidase	Male	249	41-109
	Modified jaffes reaction	Male	106	68-128
	Enzymatic sarcosine oxidase	Female	213	32-92
	Modified jaffes reaction	Female	159	60-122
Ecrcl	Enzymatic sarcosine oxidase	Male	249	36-98
	Modified jaffes reaction	Male	106	54-118
	Enzymatic sarcosine oxidase	Female	213	31-81
	Modified jaffes reaction	Female	159	58-106

Scr: Serum creatinine; Ecrcl: Estimated creatinine clearance; Rr: Reference range

subjects showed gender difference which was statistically significant for serum creatinine (p = 0.014) and estimated creatinine clearance (p = 0.018). Therefore gender based reference ranges for serum creatinine and estimated creatinine clearance using enzymatic sarcosine oxidase creatinine analytical method were constructed as shown in Table 3.

Creatinine related reference ranges established in the current study were compared with reference ranges of creatinine related parameters established using modified jaffes reaction method for a similar Kenyan population. The results were as shown in Table 4.

DISCUSION

Determination of creatinine in blood or urine specimen has been achieved by using either modified jaffes reaction or the enzymatic sarcosine oxidase methods. Clinical chemistry laboratory of Kenyatta National Hospital has been using the modified jaffes reaction method until recently when enzymatic sarcosine oxidase was introduced. It is a good laboratory practice to evaluate any new method being introduced for analytical purposes in a diagnostic laboratory in order to determine its critical reliability characteristics. The current study undertook the responsibility of establishing any analytical differences between these two creatinine analytical methods. Serum creatinine results of three hundred and sixteen study subjects which comprised of equal number of healthy individuals and renal patients were used for the comparison of the two creatinine analytical methods. The purpose of using creatinine of renal subjects was to express any analytical differences in analyzing elevated creatinine levels. On the other hand the use of healthy subjects was to express any differences in the analysis of normal creatinine levels. The study has established that when the two methods are used to determine the creatinine of the same individual, enzymatic sarcosine oxidase produces lower readings than the modified jaffes reaction method. The difference between the two methods was found to be statistically significant (renal patients: p = 0.024, healthy subjects: 0.038). Expression of this level of analytical differences requires the two methods to be considered independently as far as the interpretation of creatinine results is concerned. This expressed creatinine analytical methods difference suggests that the two methods cannot be interchangeably used. A similar study by Morimatsu *et al*. (2003), onion selective electrode and calorimetric methods of electrolytes estimation concurred with the current study findings.

The study established the interpretational tool for serum creatinine and estimated creatinine clearance reports produced using enzymatic sarcosine oxidase method. Gender differences were shown in serum creatinine (p = 0.014) and estimated creatinine clearance (p = 0.018). Therefore, specific male and

female reference ranges were established as shown in Table 3. A study by Waithaka *et al*. (2010), on a similar Kenyan study population established reference ranges for creatinine related parameters using modified jaffes reaction method. The information derived from the different reference ranges for the same parameters established using the two creatinine analytical methods indicates that the interpretation should be based on the specific reference ranges to avoid misdiagnosis of renal disorders. For example, a male serum creatinine test result of 125µmol/L produced by enzymatic sarcosine oxidase method and interpreted using jaffes reaction creatinine reference range would suggest that the client is healthy despite renal impairement clinical impression. To avoid misdiagnosis of renal disorders, it is important to interpret the results using creatinine reference ranges for the particular creatinine analytical method used.

CONCLUSION

The current study has established adult Kenyan reference ranges for serum creatinine and estimated creatinine clearance using enzymatic sarcosine oxidase analytical method. The established reference ranges are different from those of modified jaffes reaction method for the same studied adult Kenyan population. Enzymatic sarcosine oxidase method produced lower creatinine concentration than the modified jaffes reaction method for the studied parameters. Creatinine gender difference where males have higher concentration than females was evident in the current study. The two creatinine analytical methods can be used concurrently in the analysis of creatinine quoting specific reference range of the method used.

RECOMMENDATION

The established reference ranges can be used in the Kenyan health institution as opposed to literature based reference ranges. Authors recommend the establishment of reference ranges for other creatinine related parameters e.g., measured creatinine clearance not included in this study. Similar study is recommended to be carried out on children.

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