Analytical performance comparisons of Modified Jaffe’s kinetic method and Enzymatic Trinder method for creatinine along with risk zone identification

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The aim of this study is to compare analytical performance characteristics and also the patient results obtained from both Modified Jaffe’s kinetic and Enzymatic Trinder methods for serum creatinine so as to identify risk zone, if present, within the measurement range. Serum creatinine was measured on 206 left-over serum samples by Modified Jaffe’s kinetic and Enzymatic Trinder methods. For analytical performance comparisons, limit of detection (LOD), limit of quantification (LOQ), linearity, measuring range, intra and inter-assay CV were measured and compared. Statistical comparisons were done by Pearson’s correlation coefficient and Bland-Altman tests. For Enzymatic Trinder and Modified Jaffe’s kinetic methods, LODs for serum creatinine were 0.01 & 0.02 mg/dl respectively; LOQs were 0.04 & 0.06 mg/dl respectively; linearity were upto 55 mg/dl & 30 mg/dl respectively. Correlation coefficient was high (r=0.99); intra and inter-assay CV measurements were acceptable. However, CV was lower for Enzymatic Trinder method. Bland-Altman plot showed that more than 95% data points lie within ± 1.96 SD limit of mean difference value (0.16). Average discrepancy (i.e. bias) was 0.16 mg/dl across whole measurement range. However, at low concentrations, Modified Jaffe’s kinetic method gave higher values indicating systematic bias, thereby forming a “risk zone” in measurement range. Analytical performance requirements were met by both methods for routine use and good agreement exists between them. However, better performance was not shown by Modified Jaffe’s kinetic method at low concentrations. Such a “risk zone” needs to be identified by laboratories for accurate reporting of creatinine results.

INTRODUCTION

Creatinine is a non-protein nitrogenous compound and a by-product of muscle metabolism formed from creatine in a spontaneous reaction by a cyclic amide formation and removal of water (Curt et al., 2015). Serum creatinine plays a crucial role in screening for renal diseases & monitoring renal function in various diseases (Nankivell, 2001). Reliable quantification of the creatinine is the need as it has an abundant importance in various clinical conditions especially in patients of renal diseases.

Estimation of serum creatinine in a clinical bio-
chemistry laboratory can be done by various analytical methods such as isotope-dilution mass spectrometry (IDMS), High Performance Liquid Chromatography (HPLC), enzymatic methods and chemical methods. Enzymatic and Jaffe’s methods are employed in routine practice out of which the frequent one is Jaffe’s method due to its simplicity and low cost (Moore and Sharer, 2017). Jaffe’s method is known to get affected by many interfering agents and to compensate these interferences various modifications in Jaffe’s method have been proposed. Initial modification was in the form of Kinetic Jaffe’s method and later it had been further modified into Modified Jaffe’s kinetic method to further minimize the effect of interferents and improve the analytical specificity (Chung et al., 2008). Enzymatic method of creatinine estimation brings an advantage of showing more specificity than any of the forms of Jaffe’s method. Unfortunately, it is not cost-effective (ten-fold expensive than Modified Jaffe’s kinetic method) (Crocker et al., 1988). This is the reason why Jaffe’s method remains the frequently practiced method in most of the clinical laboratories especially the small- and medium-sized laboratories. It is noteworthy that the influence of interfering substances was less frequent with the enzymatic procedures, but no procedure is unaffected (Owen and Keevil, 2007; Liu et al., 2012). Several studies have already claimed about the effect of interferents and the consequent bias in Modified Jaffe’s kinetic method (Greenberg et al., 2012; Srisawasdi et al., 2010; Schmidt et al., 2015). All these reasons have sown the seeds of the necessity to compare Modified Jaffe’s kinetic method with more specific enzymatic method and identify the “risk zone”, if present in Modified Jaffe’s kinetic method. Risk zone is that area within the measurement range that is severely affected by a bias within the testing method.

In this study, we compared the analytical performance characteristics and also the patient results obtained from both Modified Jaffe’s kinetic method and Enzymatic Trinder method for serum creatinine so as to identify risk zone, if present, within the measurement range.

MATERIALS AND METHODS

This analytical study was conducted in the Clinical Biochemistry Laboratory of a medical college during the period of January 2018 to February 2018. The routine blood specimens coming to the clinical biochemistry laboratory in which sufficient serum had been left over after their routine analysis were selected as our study material. A total of 206 specimens were analyzed for our research during the study period. Hemolysed, lipemic, high bilirubin samples were excluded from the study. Since no contact with patient was made there was no role of informed consent from the patients. This is also because it is implied that the informed consent was already given previously for treatment purpose. This study was approved by Institutional Ethics Committee.

Analysis of serum creatinine was done on the same day by two different methods- Modified Jaffe’s kinetic method and Enzymatic Trinder method. Analysis of serum creatinine was done on a fully automated random access clinical chemistry analyzer. During this study period, calibration was done by using IDMS standardized, serum-based, lyophilized clinical chemistry multiconstituent calibrator provided by manufacturer of the automated analyzer while the quality control was done by using multilevel controls- erba norm (level 1) and erba path (level 2).

Methods of estimation

Principle of Modified Jaffe’s kinetic method:- Creatinine in the specimen reacts with alkaline picrate in an alkaline medium provided by sodium hydroxide to create a reddish colored creatinine picrate complex. The rate of increase in absorbance at primary and secondary wavelengths of 505 nm and 578 nm respectively is directly proportional to the concentration of creatinine in the given specimen. This reaction is non-specific as it is given by many other substances. Specificity of the method has been improved by applying kinetic method (Küme et al., 2018).

Principle of Enzymatic Trinder method:- Creatinine in the specimen is made to undergo hydrolysis by creatininease to form creatine. Creatine in turn hydrolyzed by creatinase forming sarcosine and urea. This sarcosine is made to undergo oxidation by sarcosine oxidase to form glyoxime, formaldehyde & hydrogen peroxide. Finally, in the presence of peroxidase, the hydrogen peroxide reacts with 4-aminantipyrine and N-ethyl-N-sulfopropyl-m toluidine to yield a quinoneimine dye. The resulting change in absorbance at 548 nm is proportional to concentration of creatinine in the sample (Küme et al., 2018).

For analytical performance studies, limit of detection (LOD), limit of quantification (LOQ), linearity and measuring range of the methodology were measured and compared. Also, precision analysis was done by using multi-level quality control samples (ie. erba norm (level 1) and erba path (level 2) by doing intra-assay CV and inter-assay CV measurements. Intra-assay precision was determined...
by running the quality control samples 20 times in one single analytical run. Inter-assay precision was determined by running the quality control samples successively in 20 analytical runs. Comparison studies were done by using Pearson’s correlation coefficient test and Bland-Altman test. For correlation analysis, p-value < 0.05 was considered statistically significant and highly significant if p ≤ 0.001. However, correlation strictly measures the strength of association between two variables and not the agreement between them. To study the agreement between the two methods & to assess bias across the measurement range, Bland-Altman test was performed. MedCalc statistical software version 19.6.1 was used to perform both Pearson’s correlation coefficient test and Bland-Altman test.

RESULTS AND DISCUSSION

Limit of detection (LOD):- The LOD is the lowest value for an analyte (usually expressed as concentration) that can be statistically distinguished from a blank (Armbruster and Pry, 2008; Armbruster et al., 1994; Jennings et al., 2009). Zero calibrator was analyzed 20 times to determine the LODs for both the methods. The mean LODs for Enzymatic Trinder method and Modified Jaffe’s kinetic method for serum creatinine were 0.01 mg/dl and 0.02 mg/dl respectively.

Figure 1: Scatter diagram using Pearson’s correlation coefficient test: Positive correlation between Modified Jaffe’s kinetic method and Enzymatic method

Limit of quantification (LOQ):- The LOQs were determined as the minimum concentrations at which CV were below 10% i.e. the concentration below which extrapolation is not allowed and thereby reporting can never be done (Armbruster and Pry, 2008; Armbruster et al., 1994; Jennings et al., 2009). The LOQs for Enzymatic Trinder method and Modified Jaffe’s kinetic method for serum creatinine were 0.04 & 0.06 mg/dl respectively.

Figure 2: Bland Altman plot: More than 95% data points lie within ±1.96 SD limit of mean difference (0.16)

Figure 3: Bland Altman plot showing regression line with a trend showing cluster of data points moving downward

Precision analysis was done by using multi-level quality control samples (i.e. erba norm (level 1) and erba path (level 2)). Intra-assay precision was determined by running the quality control samples 20 times in one single analytical run. Inter-assay precision was determined by running the quality control samples successively in 20 analytical runs (CLSI, 2004, 2005). As per the intra-assay and inter-assay precision data in Table 1 and Table 2, intra-assay and inter-assay CVs were lower than 2.98% (i.e. the desirable value). Also, the CV for enzymatic method was comparatively lower which indicates a better CV for enzymatic method as compared to Modified
Table 1: Intra-assay precision data

| Level       | QC 1 | QC 2 |
|-------------|------|------|
| CV(%)       | Enzymatic Trinder method | Modified Jaffe's kinetic method |
| Level 1 QC  | 1.19 | 1.45 |
| Level 2 QC  | 0.94 | 1.08 |

Table 2: Inter-assay precision data

| Level       | QC 1 | QC 2 |
|-------------|------|------|
| CV(%)       | Enzymatic Trinder method | Modified Jaffe’s kinetic method |
| Level 1 QC  | 1.71 | 2.04 |
| Level 2 QC  | 2.18 | 2.28 |

Jaffe’s kinetic method.

For correlation analysis, scatter diagram by using Pearson’s correlation coefficient test was done. Positive correlation between Modified Jaffe’s kinetic method and Enzymatic Trinder method was obtained. (See Figure 1)(r = 0.99 and p < 0.0001). For agreement between the two methods, Bland-Altman plot was done and analysed if bias is present in any of the methods. In Figure 2, more than 95% data points lie within ± 1.96 SD limit of mean difference value i.e. 0.16. This mean difference value is offset lying above zero line or line of equity. Moreover, this mean difference value of 0.16 mg/dl is the average discrepancy present across the whole measurement range. Also, for lower creatinine values, the data points are clustered above the zero line i.e. away from the zero line. In Figure 3, Bland-Altman plot shows regression line with a trend which means the cluster of data points move downward as we pass from left to right. This indicates that the difference between methods tend to get smaller (and even zero) as the mean value increases. The systematic bias (or mean bias) is more for smaller creatinine values.

Jaffe’s method is known to get affected by various non-creatinine chromogens and so for minimizing interferences, various modifications of Jaffe’s method are being used in clinical diagnostic laboratories; one of these is Modified Jaffe’s kinetic method (Chung et al., 2008). Specificity of enzymatic method is a known attribute contributing to non-interference/less interference by the interfering compounds. Therefore, Enzymatic Trinder method for serum creatinine is considered to be more accurate and precise as compared to Modified Jaffe’s kinetic method (Crocker et al., 1988).

In this study, we compared the analytical performance characteristics and the patient results obtained from both Modified Jaffe’s kinetic method and Enzymatic Trinder method for serum creatinine so as to identify risk zone, if present, within the measurement range.

The mean LODs for Enzymatic Trinder method and Modified Jaffe’s kinetic method for serum creatinine were 0.01 mg/dl and 0.02 mg/dl respectively. The LOQs for Enzymatic Trinder method and Modified Jaffe’s kinetic method for serum creatinine were 0.04 & 0.06 mg/dl respectively. For both the methods, we performed linearity tests and it was found that Enzymatic Trinder method was linear upto 55 mg/dl while Modified Jaffe’s kinetic method showed linearity upto 30 mg/dl. So the measuring range for Enzymatic Trinder method was 0.04 - 55 mg/dl while the measuring range for Modified Jaffe’s kinetic method was 0.06 – 30 mg/dl. Thus, for creatinine measurements in patients, a sufficiently wide window is provided by both the methods.

The maximum acceptable CV i.e. desirable imprecision performance must be less than one-half of intra-individual variations (Fraser et al., 1997; Fraser and Petersen, 1999). From various biological variation studies, it has been deduced that intra-individual CV for serum creatinine is 5.95%, so maximum acceptable CV is 2.98% (Ricós et al., 1999). In our study, the intra-assay and inter-assay CVs were found to be lower than 2.98%. Also, the CV was better for enzymatic method as compared to Modified Jaffe’s kinetic method. This indicates better precision and thereby better analytical performance for the Enzymatic Trinder method which could be due to more specificity provided by the enzymatic method. It is noteworthy that CV was considerably on the lower side for both the methods which indicates that they show better analytical performance characteristics thereby making both the methods acceptable (even if Modified Jaffe’s kinetic method is less specific than Enzymatic Trinder method) for routine use. However, practical acceptibility of Modified Jaffe’s kinetic method is more due to its low cost (ten-fold cheaper) as compared to Enzymatic Trinder method.

Paired data was then obtained by comparing the
patient’s results obtained from both Modified Jaffe’s kinetic method and Enzymatic Trinder method. In our study, positive correlation obtained between Modified Jaffe’s kinetic method and Enzymatic Trinder method is in accordance with previous studies conducted by Kume et al. (2018); Gencheva and Ruseva (2015). Correlation quantifies the degree to which the two variables are associated. However, high correlation does not mean that there is good agreement between the two methods. It can be misleading to assess agreement by using correlation, regression etc. because they evaluate only linear association between the two variable sets and not the agreement between them (Schober et al., 2018). For agreement between the two methods, Bland-Altman test was done and analysed for bias, if present in any of the methods.

Bland and Altman recommended that 95% of the data points should lie within ±1.96 SD of the mean difference, if the differences are normally distributed (Giavarina, 2015). In our study, in the Bland Altman plot, approximately 95% of the data points lie within ±1.96SD of the mean difference indicating that a good agreement exists between the two methods. Also, by representing every difference between two paired methods against the average of the measurement and plotting difference against mean, the Bland-Altman plot allows detection of any possible relationship between true value and measurement error. The bias is computed quantitatively as the difference between the average value determined by two methods. If it is close to zero, then bias doesn’t exist. However, if it is not close to zero, then it indicates that the two assay methods are producing different results systematically i.e. systematic bias (Giavarina, 2015).

Bland-Altman plot needs to be assessed visually (Giavarina, 2015). In this study, for lower creatinine values, the data points are clustered above the zero line i.e. line of equity which means that the differences are not close to zero indicating systematic bias (or mean bias). In our study, for higher creatinine values, the data points are close to the zero line i.e. line of equity which means that the differences are close to zero. This is the first point that is required to evaluate the agreement between the two methods and so it is concluded that the two methods are essentially equivalent for higher creatinine values. Both the scenarios conclude that variation of atleast one of the methods (i.e. Modified Jaffe’s kinetic method in our study) depends strongly on the magnitude of measurements. The mean value of Modified Jaffe’s kinetic method is 1.27 mg/dl which is higher than the mean value of Enzymatic Trinder method (1.05 mg/dl). So it can be deduced that Modified Jaffe’s kinetic method over-estimates and gives a higher value for lower creatinine levels when compared to Enzymatic Trinder method but shows good agreement at higher creatinine values. Thus, the discrepancy (or variability) in measurement values between the methods do exist across the measurement range and largely at lower creatinine values causing systematic bias. This is also supported by presence of regression line with a trend. Systematic bias is the dominant phenomenon playing a role in Modified Jaffe’s kinetic method at lower creatinine values. Bias is the average discrepancy (or variability) across the whole measurement range between the methods (Giavarina, 2015) and is interpreted by the mean difference value which in our study is 0.16 mg/dl. However, since this discrepancy of 0.16 mg/dl is to be interpreted clinically and not statistically, we can safely interpret that for creatinine parameter, a discrepancy of 0.16 mg/dl could be considered as a clinically allowable discrepancy because average discrepancy of 0.16 mg/dl is not large enough to be important in most of clinical scenarios. Consequently, it is to be noted that systematic bias exists only for lower creatinine values while the average discrepancy between the methods across the whole measurement range is clinically permissible. This generates the need to focus scientifically on lower creatinine ranges that seems to be an area of concern for Modified Jaffe’s kinetic methodology.

It is thereby suggested that risk zone identification of Modified Jaffe’s kinetic method could be done to identify the creatinine ranges where the variability matters and where not. In this study, it can be observed that any creatinine value less than 2 mg/dl is the “risk zone” because the average discrepancy when creatinine is less than 2 mg/dl is higher. Risk zone identification can be done by plotting the Bland-Altman plot with regression line’s trend as done in this study. If a patient’s result is outside the “risk zone” (i.e. more than 2 mg/dl in our study) then the laboratories can proceed with the reporting of the test result. However, for the results that are falling in the risk zone, then the more specific enzymatic method could be used further for accurate reporting of results. Such practice must be followed by the laboratorians especially in some special circumstances such as renal transplant patients, GFR estimation or on insistence of clinician. Although highly accurate and specific, the enzymatic method is very expensive as compared to the Modified Jaffe’s kinetic method making it probably a tedious task for small- and medium-sized laboratories to identify the risk zone in the measurement range. High cost of enzymatic kits becomes the...
only reason why complete abolishment of Modified Jaffe’s kinetic method could never be considered. Systematic bias is unaffected by sample size. There is only one strategy that can be applied to decrease and nullify the effect of systematic bias: Review, criticize and modify testing procedure. (Abimanyi-Ochom et al., 2019). The authors would like to stress upon the fact that further reviewing, critical appraisal and modification in the testing procedure of Modified Jaffe’s kinetic method is the need of an hour amongst the cohort of laboratory scientists and technicians. It is hereby recommended that a boost in the research is required to study, innovate and further improve Modified Jaffe’s kinetic method of creatinine estimation.

CONCLUSION

This study concludes that there exists a good agreement between Enzymatic Trinder method and Modified Jaffe’s kinetic method and both the methods meet the analytical performance requirements for routine use. However, Modified Jaffe’s kinetic method overestimates the creatinine values at lower ranges, thereby indicating the presence of “risk zone” in its measurement range.

ACKNOWLEDGEMENTS

The authors are thankful to all who have extended their support for the completion of this study.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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