Comparison of Jaffe and enzymatic methods for creatinine estimation and their effect in GFR calculation in a tertiary care hospital

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ABSTRACT

Introduction and Aim: Serum creatinine (SCr) and estimated glomerular filtration rate (eGFR) gives the idea of overall functional status of kidney. Measurement of SCr by different method and discordant result between them can misclassify different stages of chronic kidney disease (CKD). Many of the tertiary care health centre, SCr measured by Jaffe method is still the method of choice because of its cost effectiveness even if it is more susceptible for interference. Our aim was to measure SCr level by both Jaffe’s & enzymatic method and analysing the discordance rate of eGFR and their effect in staging of kidney disease in CKD patients.

Materials and Methods: In this observational study 330 serum sample were analysed for creatinine by Jaffe’s and enzymatic methods in a fully automated analyser using commercially available kits. Modification of Diet in Renal Disease (MDRD) formula was used for estimating GFR.

Results: In 330 subjects eGFR values calculated on incorporating SCr were found as 58.84 ± 68.34 (median (IQR) = 26.67(15.09-90.84) and 44.49 ± 41.18 (Median (IQR) = 26.86 (14.69-67.69) ml/min/1.73 m² respectively by Jaffe’s and enzymatic method. Concordance correlation coefficient between two methods for SCr was statistically significant. Bland-Altman plot showed with increasing value of SCr, the difference between the SCr values given by these two methods increased. Jaffe’s creatinine has lower value in comparison to enzymatic Cr. There was a significant difference between eGFR obtained from SCr estimated by Jaffe’s and enzymatic method.

Conclusion: All laboratory should use uniform method for creatinine estimation. Enzymatic method for creatinine estimation should not be compromised over cost of test, so that less variability in creatinine result can lead to more accurate staging of the disease.

Keywords: Analytical variation; glomerular filtration rate; method comparison; renal dysfunction.

INTRODUCTION

Renal function can be assessed by estimation of glomerular filtration rate (GFR). Measurement of serum creatinine (SCr) has been used as a cost effective and endogenous marker, to assess glomerular function since decades. Indeed, SCr measurement is incorporated into the equation designed to estimate GFR (eGFR) (1). Calculated GFR value is superior to SCr alone, as the later might be influenced by many biological factors like age, gender, ethnicity, muscle mass, nutritional status of the individual and the analytical method it is being estimated (2-4). Primarily eGFR calculation is done to identify the presence of renal diseases and is been reported to be useful for determination of different stages of CKD and also to monitor response to treatment (5). SCr is commonly
measured on automated analyser using either Jaffe’s method or enzymatic assay. Indeed, Jaffe’s method is more susceptible to interferences like bilirubin, ketone bodies, protein etc. Whereas enzymatic assay for SCr exhibits comparatively less interference, both in frequency and degree of interference (6). Such type of interference, that either increase or decrease the creatinine value, will consequently affect eGFR calculation and misclassification of CKD. Therefore, several authors have also suggested to abandon Jaffe’s assay and are in favour of enzymatic assay (7). Though Jaffe’s method is subjected to bias due to interfering substances and loss of analytical specificity, still it is most commonly used in clinical practice, as it is less expensive compared to enzymatic method.

**eGFR and Creatinine relationship**

Analytical bias in SCr measurement gives unreliable renal functional status, incorrect staging and inappropriate treatment modality. Inter-laboratory variations in SCr measurement was observed worldwide because of different method of estimation. Two methods are most widely used by the lab professionals for measuring SCr and urinary creatinine. Jaffe’s alkaline picrate method was initially developed in the year of 1886. This method is interfered positively and negatively by number of chromogens such as bilirubin, cephalosporin, ketone bodies, glucose, protein, etc. (14), but still remain as most commonly used method. Over the year for overcoming these interference this method has undergone many modifications. Now compensated Jaffe (adjusted for protein interference) is used. In Enzymatic creatinine assay overall influence of interfering substance is less but is not used by clinical laboratory because of its high cost (15). Accuracy in the test result, cost of the test, ability to differentiate between the states of diseases, decision in treatment modality should be taken care while evaluating the method comparison studies. Therefore, the present study was attempted to analyse the discordance rate in eGFR value based on serum creatinine measurement by both Jaffe’s method and enzymatic method. To compare the influence of creatinine methodologies on eGFR calculation and to see whether low cost Jaffe’s creatinine assay still can be used safely in renal diseases or should be discarded.

**MATERIALS AND METHODS**

The present observational study was conducted in central diagnostic laboratory (Biochemistry section) of a tertiary care institute, over a period of six months (1st August 2018 to 31st January 2019). 330 participants who came for renal profile analysis were explained about their leftover serum sample analysis for SCr by different method in the same laboratory. Informed consent was obtained from them and explained about less than minimal risk is
involved. Pregnant women were excluded from the study.

Left over serum samples were processed on the same day of collection for SCr. It was analysed by both Jaffe’s alkaline picrate and enzymatic method in fully automated clinical chemistry analyser, using commercially available kit as per manufacturer’s protocol. Internal Quality control (IQC) and External Quality Assurance Service (EQAS) was monitored for SCr regularly.

Kinetic Jaffe reaction without de-proteinization compensated for serum creatinine based on the principle of yellow-red coloured alkaline picrate formation. The rate of dye formation is directly proportional to creatinine concentration in the sample.

In enzymatic assay creatinine undergoes series of reactions. Initially it is converted to sarcosine and urea by creatine amidinohydrolase. Sarcosine is oxidised to glycine, formaldehyde and hydrogen peroxide by sarcosine oxidase. The final reaction involves peroxidise- catalysed oxidation of a leuco dye to produce coloured product and absorbance is measured.

Information regarding age, gender, height, weight was recorded and GFR was calculated by using 4 variables MDRD formula as follows

\[ \text{GFR} = \text{175} \times \text{[SCr]}^{-1.154} \times \text{[age]}^{-0.023} \times 0.742 \times 1.212 \text{ (If female)} \times 1.212 \text{ (If black)} \]

**Statistical analysis**

The data was analysed by Statistical Package for the Social Sciences (SPSS) software version 15.1. Quantitative data was described using mean and standard deviation as well as median (Inter Quartile range) for the variables having skewed distribution. Bland-Altman plot (17) was used to assess the agreement in Serum Cr and eGFR values obtained using Jaffe and enzymatic methods. Concordance correlation was also performed to assess the degree of concordance between these two methods. A p-value less than 0.05 was considered as statistically significant.

**RESULTS**

The present study included 330 subjects with a mean age of 57±18 years. Among these 108(32.7%) were female. Average value of SCr obtained by Jaffe and enzymatic method for 330 patients were 2.93±2.28 (Median (IQR)= 2.3 (1.1- 4.0) and 2.83±2.41 (Median (IQR)=2.19 (0.82-3.99)) respectively. eGFR calculated on the basis of SCr values were found as 58.84±68.34 (Median (IQR)= 26.67 (15.09-90.84)) and 44.49±41.18 [Median (IQR)= 26.86 (14.69-67.69)] respectively by Jaffe and enzymatic methods.

The concordance correlation coefficient between Jaffe and enzymatic method for creatinine was very high i.e. 0.987(0.984-0.989) which was statistically significant.

![Fig.1 Bland -Altman agreement plot of SCr value between Jaffe and Enzymatic method](image)

The Bland-Altman plot (Fig.1) showed that with increasing value of serum Cr, the difference between the serum Cr values given by these two method increased. In this case Jaffe’s creatinine has lower values in comparison to enzymatic Cr. However, for lower Serum Cr level, it is reversed. Overall difference between SCr given by these two method is 0.097(-0.631-0.825).The similar results i.e. slightly higher value for SCr in Jaffe’s method was depicted for lower SCr and slightly lower value was depicted in Jaffe’s method in comparison to enzymatic method for higher SCr level in concordance graph (Fig.2).

Such exploration indicated that though the magnitude of concordance correlation was very high, there is a poor agreement between the two methods.
Table 1 showed that difference of Jaffe to enzymatic SCr which is significantly higher (Jaffe > Enzymatic) for lower SCr i.e. SCr< 1.5 mg/dL. This difference decreased with increasing SCr value i.e. for group II & III but it was still statistically significant. However, for group IV (SCr > 4 mg/dl), on an average enzymatic values were slightly higher than Jaffe value but not statistically significant.

eGFR values were calculated on the basis of SCr value obtained by two methods.

Table 2 showed the distribution of CKD staging. A total of 267 out of 330 subjects, 80.9% were classified into same stage by both the methods. Both methods have classified 54 patients as normal. However, by Jaffe method 55 patients were classified as normal and by enzymatic method 84 patients were classified as normal. But out of these 84 patients, 28 patients were classified into stage I and 2 patients were classified as stage II by Jaffe’s method.

Among the 330 patient, only 7(2%) were classified to lower staging by Jaffe in comparison to enzymatic. However, 56 (7%) were classified to higher staging by Jaffe in comparison to enzymatic especially for higher eGFR. Concordance correlation for eGFR observed between these two methods is 0.808(95% CI: 0.785-0.830) (Fig.3).

Table 1: Mean Difference between Jaffe and Enzymatic method of SCr estimation in four different creatinine range.

<table>
<thead>
<tr>
<th>Groups</th>
<th>No. of observations (n)</th>
<th>Mean difference of two methods ±SD (mg/dL)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (SCr ≤ 1.5mg/dL)</td>
<td>120</td>
<td>0.18±0.09</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Group II (1.5-2.5 mg/dL)</td>
<td>66</td>
<td>0.14±0.22</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Group III (2.5-4 mg/dL)</td>
<td>62</td>
<td>0.07±0.24</td>
<td>0.018</td>
</tr>
<tr>
<td>Group IV (≥ 4 mg/dL)</td>
<td>82</td>
<td>-0.05±0.65</td>
<td>0.542</td>
</tr>
<tr>
<td>Total</td>
<td>330</td>
<td>0.10±0.37</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Foot notes: SCr- Serum creatinine, *- P <0.001 indicates that significant difference between Jaffe and Enzymatic method of SCr estimation, when level is <1.5 and 1.5-2.5 mg/dL.

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Bland-Altman plot (Fig.4) to assess the agreement between Jaffe and enzymatic Cr for eGFR values showed the mean, lower and upper limits for Jaffe-enzymatic Cr, are -14.35, -78.10 and 49.41 respectively. The observed correlation between difference of eGFR values by two methods and mean eGFR was -0.846 which was statistically significant. This happened mainly as previously explained that the mean SCr level with Jaffe method was significantly higher compared to enzymatic method for lower creatinine values but lower for high creatinine values (Table1). Such negative correlation indicates about the poor agreement between Jaffe and enzymatic method, though there is a good concordance correlation coefficient between Jaffe and enzymatic method for creatinine.

DISCUSSION

CKD is one of the public health problems worldwide. GFR, being estimated by using different formula is the best index to evaluate renal function and CKD staging instead of SCr alone. MDRD formula has been widely used and validated in CKD patients to predict GFR by incorporating SCr value (18). In clinical laboratory Jaffe’s and enzymatic method are the two most common methods used for measuring SCr. Till date, several authors have attempted the comparison study between the above two methods in specialized populations (2, 19) and it was limited to only analytical performances. Information regarding in accuracy of SCr measured by different methods and impact of bias in clinical staging of the disease is our source of concern. The purpose of this study was to evaluate and compare the analytical performance of the SCr measured by Jaffe’s and enzymatic method in a tertiary care health institute, where in spite of large sample load, still Jaffe’s method preferred because of its economy. As analytical performance is the most fundamental criteria for comparison study, we have analyzed Bland-Altman plot to assess the agreement for eGFR calculation derived from both methods. We observed a significant difference between calculated values of eGFR by two different methods (Fig.4), which was significantly correlated to the mean eGFR. This agrees to a similar kind of study by Qiet al., (20).Thus the magnitude of difference between two methods on a single sample was significant relative to analytical variation, which can lead to misclassification. The most frequent incidence of significant misclassification in our data could be attributed to use of Jaffe’s test (7%) in comparison to enzymatic method, especially for higher eGFR. However, the risk of misclassification may vary depending on the relative proportion of patient to be screened & probability of patient outcome. So it is not sufficient to evaluate the risk on the basis of misclassification, as it might not affect outcome. Looking at the concordance correlation between the two methods, 0.808 (0.785-0.830) in our study, it can be assumed that Jaffe’s method could be cost effective in a population where clinical risk associated renal status is low. Several data have been published giving a significant difference in magnitude relative to biological variation (21). There are still concerns for misclassifying patients of renal failure due to
method non-specificity (22). The overall risk associated with Jaffe’s method depends on probability and consequence of misclassification. Significant higher result was documented (Jaffe’s method > enzymatic method) for low SCr in Table1, which agrees to evaluation by Kume et al., (23). This suggests that the use of method based on enzymatic reaction could improve the specificity of measurement (11). We have also found the negative correlation between differences of eGFR by 2 methods and mean GFR. It was found to be statistically significant (- 0.84). So it is important to note that eGFR has an uncertainty, which varies with the level of SCr (24). This biological variation definitely will have an impact on the interpretation of CKD staging (6).

CONCLUSION

GFR calculation is widely used to diagnose & monitor CKD. It is subject to variation because of analytical error of SCr measurement and biological variation. Present comparison study was an attempt to analyse the discordance rate in eGFR based on serum creatinine measurement obtained by both Jaffe’s and enzymatic methods. Existing data observed a considerable overestimation of eGFR in compensated Jaffe’s method in individuals with low SCr than enzymatic method. In enzymatic method more subjects falling under normal (84 subjects) category were more and at the same time by Jaffe’s method 55 subjects were normal. In our study concordance correlation for SCr. and eGFR by both the methods was good but agreement between the two was poorly observed. Both Jaffe’s method and enzymatic method could meet requirement in routine use so far as analytical performance is concerned. As we know, bias due to measurement difference is the most important factor that affects misclassification, so this problem could be overcome choosing correct method of analysis. Both biological variations as well as analytical imprecision should be taken into account to avoid uncertainty of measurement of eGFR. Such exploration will help in decision making of universal adoption of a particular method to get more accurate staging and accordingly management of the disease process. Such finding will help to evaluate clinical effectiveness and associated clinical risk in CKD patients. Simultaneously outlay can be reduced if bulk will be tested and all the laboratories may have one method for estimation.

Limitations

Our study was limited only to analytical performance. Effect of interference and different clinical conditions other than CKD was not included in this study. A study on larger group must be warranted.

References


