Estimation of Serum Creatinine by Routine Jaffé’s Method and by Dry Chemistry in Icteric and Hemolytic Serum Samples

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ABSTRACT

Background: Newer analytical methods are introduced in clinical biochemistry laboratory for the purpose of improvement of quality, automation, to reduce the cost or to simply measure a new analyte. Serum creatinine is one of the renal function test. Creatinine is measured in serum by modified Jaffé’s method which involves wet chemistry (SCRMJ) and the new dry chemistry which utilizes the microslides and involves enzymes (SCRMS). Various kits available commercially which are based on enzymatic methods to overcome the shortcomings and problems inherent in the Jaffé’s method. Aim: The aim of the present study is to compare the results of creatinine estimation by modified Jaffé’s method (SCRMJ) or wet chemistry and dry chemistry (SCRMS) in icteric and haemolytic serum samples. Methods and Materials: Forty serum samples each of icterus and haemolysis were analyzed by modified Jaffé’s method (SCRMJ) (wet method) and patented dry chemistry (SCRMS) method developed by ortho clinical diagnostics. Results: The Creatinine concentration in serum is comparatively lower when estimated by dry chemistry (SCRMS) developed by ortho clinical diagnostics on Vitros 250 analyzer as compared to modified Jaffé’s method (wet chemistry). The values of creatinine are found to be both accurate and precise by the enzymatic method in icteric and haemolytic serum samples. Conclusions: Dry chemistry eliminates the possibility of overestimation of creatinine in icteric and haemolytic serum samples and estimate the true value of creatinine in serum for better treatment planning.

Keywords: Icteric, haemolytic, creatine, creatinine, interference

INTRODUCTION

Clinical biochemistry laboratories endeavours to introduce new analytical methods, to improve the existing methods introducing better quality in terms of accuracy and precision over existing methods, to measure a new analyte required for diagnosis and prognosis [1]. Creatinine is a catabolic product formed from creatine phosphate. Serum creatinine is utilized as a screening test in the clinical evaluation of renal function [2]. Serum creatinine levels are influenced by creatinine filtration rate in kidneys, sex, age, muscle mass and the analytical method utilized for measurement [3-6]. Creatinine is measured by a colorimetric method in blood and urine invented by Max Jaffé (1841-1911) in 1886 based on Jaffé reaction in clinical chemistry [7]. Jaffé discovered that on reaction with sodium hydroxide and picric acid solution, creatinine formed a reddish orange colour which can be measured by spectrophotometer [8]. The Jaffé’s method has progressed gradually through many phases over the years. Earlier methods involved the use of deproteinized blood. Later creatinine was isolated from common interfering substances by adsorption on aluminium silicate such as Lloyd’s reagent, followed by elution and later treated with into alkaline picrate solution [9]. This improved the specificity of Jaffé’s method. Cation exchange resins were also utilized for this purpose. The other strategy used was to estimate creatinine by Jaffé method at both alkaline pH and after acidification to a more neutral pH. Since only interfering substances react at neutral pH, by the difference a more accurate result for creatinine could be established [10]. The era of automation which began in 1957, incorporated on-line dialysis to remove protein [11], an important interfering substance in the Jaffé ‘s assay [12]. Protein bound interfering substances such as bilirubin was removed but
smaller molecules such as glucose, pyruvate, acetoacetate, and cephalosporin were still able to cross the membrane and produce false high creatinine results on Technicon flow auto analyser invented by Leonard Skeggs [13].

With the progressive introduction of random access, centrifugal and other discrete analysers, the specificity was achieved in the absence of dialysis by careful monitoring of the kinetics of the reaction of creatinine and interfering substances reacting with alkaline picrate. It was reported that the reaction of creatinine by Jaffé reaction progressed in the presence of both fast and slow reacting interfering substances [10, 14, 15].

Enzymatic method for creatinine estimation was developed to overcome the problems inherent in Jaffé’s method. The underlying principle involves enzyme-catalysed series of steps in reaction which results in formation of hydrogen peroxide. A Trinder indicator system is the final step in the reaction sequence, resulting in an intense red colour with maximum absorbance at wavelength of 510 nm [16].

The Vitros slides are dry, multi-layered analytical elements coated on polyester supports. The slide is composed of several layers such as spreading layer, scavenger layer, reagent layer (s) and plastic or support layer. A small aliquot of serum deposited via automation on the slide and evenly distributed to all the layers. The spreading layer contains appropriate substrate and components for reaction. The rate of change measured by reflectance spectrophotometry, optical density of coloured complex formed is directly proportional to the concentration of creatinine in serum [17]. The Vitros 250 developed by Ortho clinical diagnostics which is a dry chemistry system involves enzymatic reagent system for the estimation of creatinine.

**METHODS**

The present study is an experimental study to compare the analytical methods to estimate serum creatinine. Forty serum samples each hyperbilirubinemia and haemolysis were received in central clinical laboratory, biochemistry division were used for analysis. The serum creatinine was estimated from each sample by dry chemistry on Vitros 250 analyser developed by Ortho clinical diagnostics and further also by wet chemistry by Jaffé Method [16]. Serum samples belonging to patients on various drug treatments were excluded from the study.

**Ethics**

Institutional ethical committee and granted approved permission (PIMS/RMC/2015/104).

**Statistics**

Test of significance, paired t-test applied, with p<0.05 considered as significant.

**RESULTS**

In the present study, the Tables 1 and 2 depict that there is an overestimation of serum creatinine values when estimated by modified Jaffé’s method or wet chemistry (SCrMJ) in icteric and haemolytic serum samples. The results are comparatively lower when estimated by dry chemistry or enzymatic method (SCrMS) developed by Ortho clinical diagnostics on Vitros 250 analyser (Figures 1 and 2). The values of creatinine are found to be both accurate and precise by the enzymatic method in icteric and haemolytic serum samples (Figures 3 and 4).

<table>
<thead>
<tr>
<th>Serum total bilirubin in mg/dl</th>
<th>Number of patients</th>
<th>Serum creatinine in mg/dl</th>
<th>Dry Chemistry Mean ± SD (SCr MS)</th>
<th>Wet Chemistry Mean ± SD (SCr MJ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0-2.0</td>
<td>14</td>
<td>1.21 ± 0.37</td>
<td>1.31 ± 0.52</td>
<td></td>
</tr>
<tr>
<td>2.1-3.0</td>
<td>13</td>
<td>1.20 ± 0.37*</td>
<td>1.29 ± 0.35*</td>
<td></td>
</tr>
<tr>
<td>3.1-4.0</td>
<td>4</td>
<td>0.85 ± 0.25</td>
<td>0.96 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>4.1-5.0</td>
<td>9</td>
<td>2.40 ± 0.37</td>
<td>2.51 ± 0.47</td>
<td></td>
</tr>
</tbody>
</table>

*Test of significance of difference by paired t-test is statistically significant, p<0.05.
Table 2 serum creatinine in haemolytic samples done by dry and wet chemistry

<table>
<thead>
<tr>
<th>% Hemolysis</th>
<th>Number of patients</th>
<th>Serum creatinine in mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Dry Chemistry Mean ± SD (SCr MS)</td>
</tr>
<tr>
<td>Oct-50</td>
<td>3</td>
<td>0.47 ± 0.20</td>
</tr>
<tr>
<td>51-100</td>
<td>19</td>
<td>0.44 ± 0.17*</td>
</tr>
<tr>
<td>101-150</td>
<td>10</td>
<td>0.94 ± 0.25</td>
</tr>
<tr>
<td>151-200</td>
<td>8</td>
<td>0.54 ± 0.32</td>
</tr>
</tbody>
</table>

*Test of significance of difference by paired t-test is statistically significant, p<0.05.

The different analysis showed an agreement between the two methods within the studied range. The mean difference between two methods for icteric samples was 0.091 mg/dL (Figure 5) for haemolytic samples and for -0.25 mg/dL (Figure 6).

![Figure 1 Comparison of Scr (MJ) and Scr (MS) in Icteric samples](image1.png)

![Figure 2 Comparison of Scr (MJ) and Scr (MS) in haemolytic samples](image2.png)
Figure 3 Passing Bablock regression between Scr (MJ) and Scr (MS) in Icteric samples

Figure 4 Passing Bablock regression analysis between Scr (MJ) and Scr (MS) in haemolytic samples

Figure 5 Different plot analysis showed a mean between Scr (MJ) and Scr (MS) in Icteric samples
Padmanabhan Preeti, et al.

Estimation of estimated creatinine clearance (eCcr) on the basis of serum creatinine, by Cockcroft-Gault formula [18].

Consider a woman of 60-year age and 65 kg body weight (b.w.) with serum creatinine estimated as 2.1 mg/dL by wet chemistry and 1.8 mg/dL by dry chemistry.

Therefore, eCcr by dry chemistry = \((140\text{-age}) \times b.w (kg) \times 0.85\) for females \(\div 72\times\) serum creatinine (mg/dl by dry chemistry) = \((140-60) \times 65 \times 0.85 \div 72 \times 1.8=4420 \div 129.6=34.10 \text{ ml/min.}\)

eCcr by wet chemistry = \((140\text{-age}) \times b.w (kg) \times 0.85\) for females \(\div 72\times\) serum creatinine (mg/dl by wet chemistry) = \((140-60) \times 65 \times 0.85 \div 72 \times 2.1=4420 \div 151.2=29.23 \text{ ml/min.}\)

It is evident from the example considered that the values of serum creatinine are inversely related to the estimated clearance of creatinine. Accuracy and precision is maintained when serum creatinine is estimated by dry chemistry as compared to wet chemistry which gives a higher value for the same patient considered. Serum creatinine have an impact on the values of estimated creatinine clearance that means a higher value of estimated creatinine clearance maybe expected by dry chemistry than when calculated by wet chemistry. This has a major role in determining the correct drug dosages for renal patients. The advantage of adopting dry chemistry for estimating serum creatinine has further a role in the cases of borderline patients suspected to suffer from renal diseases.

The practice of using dry chemistry would guarantee exact clinical idea about the clearance of creatinine at the glomerulus which is rather underestimated when the wet chemistry is used for serum creatinine estimation. All these advantages ascertain that dry chemistry is superior to wet chemistry in the estimation of creatinine in serum.

**DISCUSSION**

In the study, we have compared the performance characteristics of Scr (MJ) and Scr (MS) in hemolytic and icteric samples. The mean Scr (MS) was found to be lower than the mean Scr (MJ) in all stages of hemolytic as well as icteric samples. This is clear indication that dependence of Scr (MJ) could lead a false diagnosis of the renal function. Similar results have been reported by Sayal, et al. who found haemolysis, lipaemia and icteric affects the accuracy of creatinine evaluation [19]. This rise in Scr (MJ) could also be due to release of chromogens by haemolysis which increase the resultant values of creatinine falsely. The NKDEP concluded that the performance of the Jaffé method is compromised by analytical non-specificity. It was reported, as much as a 30% bias at concentrations <1 and up to 10% at levels >1 mg/dL is possible [20]. There was a good correlation between Scr (MJ) and Scr (MS). However, the passing Bablock regression (r value) for haemolytic samples was 0.741.

Our findings are in agreement with Ou, et al. [21] who found that there was a significant mean bias between Scr (MJ) and Scr (MS) methods. They confirmed that the Scr (MJ) method was more susceptible to interferences than the enzymatic method.
Both Jaffé’s method and enzymatic method of creatinine estimations have major disadvantage of positive interference by endogenous substances [22-24]. High concentrations of bilirubin [25,26] and glucose [27,28] are the main interferents in the Jaffé’s method. High concentrations of glucose, ammonia interfere significantly with creatinine estimated by enzymatic method [29,30].

The Jaffé’s reaction is observed to be non-specific and causes falsely elevated creatinine results in the presence of protein, glucose, acetoacetate, ascorbic acid, guanidine, acetone, cephalosporin, aminoglycosides mainly streptomycin, ketone bodies, α-keto acids and other organic compounds [7,19].

Artefacts such as haemolysis, lipaemia and icteremia can also affect the accuracy of creatinine evaluation. Haemolysis releases chromogens which also enhances the resultant values of creatinine falsely [19].

The exact mechanism of bilirubin interference in Jaffé’s method is not known but the colour of bilirubin affects the spectrum absorption with yellow colour of picrate used in creatinine estimation. In case of icteric samples when creatinine value is to be established, the colour produced due to bilirubin should be removed or minimized. This is commonly done by oxidation of bilirubin to biliverdin by oxidizing agents. In a recent study by Chaudhary et al. the oxidation of bilirubin is carried out by preincubation with NaOH before estimation of creatinine [3].

Interference by paraproteins in IgG myeloma patients showed low values of serum creatinine by Jaffé’s method but expected results by enzymatic methods [31]. Acid precipitation or dithiothreitol preincubation removed the artefact in creatinine estimation [32].

The Jaffé’s method is vulnerable to chromogens such as cephalosporin and ketones. A bichromatic rate technique at 510 nm as well as 600 nm is adopted to establish the creatinine concentration in order to abolish typical interferents. The Jaffe’s method may include potassium ferricyanide, which oxidizes bilirubin thus reducing interference from icterus. Hyperproteinaemia (hypergammaglobulinaemia, hyperalbuminemia) may falsely increase creatinine values whereas hypercholesterolemia and presence of dextran decreases creatinine values [33].

The mechanism of action of interferents like glucose and bilirubin is that both inhibit the reaction between creatinine and alkaline picrate [14,34]. Acetoacetate and cefoxitin directly react with alkaline picrate. Acetoacetate reacts at rate faster as compared to creatinine with picrate [35]. The thiophen nucleus is the active moiety in cefoxitin molecule which reacts with Jaffe’s reagent [36].

Cephalosporin have been reported to depict a significant positive increase in creatinine concentrations by kinetic Jaffé’s method but does not affect in the enzymatic assay of creatinine [36].

Enzymatic method of creatinine measurement is considered to be more specific, it may also face interference problems. Bilirubin and assay substrate for hydrogen peroxide competed for each other, leading to underestimation of creatinine. This problem was overcome by an efficient hydrogen peroxide acceptor (tri-iodo-hydroxy-benzoic acid) and include potassium ferricyanide and detergents to reduce bilirubin interferences [13].

Serum creatinine (MS) by reflectance spectrophotometric method, Micro-slide technology using enzymatic method has been proposed as a sensitive parameter for assessing renal function. Scavenger layer of micro slide removes the ascorbic and uric acid interferences. Spreading layer of micro-slide is protecting from protein and icteric interferences. Also Scr (MS) is excellent correlation with IDMS reference method. The advantage of this enzymatic method of creatinine estimation by dry chemistry on Vitros is the minimization of bilirubin and hemoglobin interferences by the retention of the interferents on the spreading layer of the slide used.

CONCLUSION

The Dry Chemistry based on enzymatic method of creatinine estimation is considered superior to the routine Jaffé’s method or Wet chemistry for measurement of creatinine as indicated by results in icteric and haemolytic serum samples.

ACKNOWLEDGEMENTS

We are thankful to:

1) Dr. N. Krishnamurthy, Lab Support Specialist, Ortho Clinical Diagnostics, Nagpur for statistical analysis and technical discussion.

2) Staff of Biochemistry division of Central Clinical Laboratory, PIMS-DU.
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