The Effects of Both Glucose and Bilirubin on the Jaffe’s Kinetic and Enzymatic Methods for Creatinine Measurement”

Abdulrahman Hefdhallah Amer 1 & Dr. N. Haridas 2
1Ph.D. Research Scholar, Medical Lab. Technology, Pramukhswami Medical College, Karamsad. Sardar Patel University, V. V. Nagar Anand, Gujarat, India Pin Code- 388325.
and Thamar University-Yemen.
2Dr. N. Haridas (Ph.D. Guide), Professor, Biochemistry Pramukhswami Medical College, Karamsad Anand, Gujarat, Pin Code: 3883255

ABSTRACT:
Background: The Jaffe’s and enzymatic methods are the two most common methods used for measuring serum creatinine. Interferences with other substance can lead to misdiagnosis. In this study, the objective was to compare the enzymatic and Jaffe’s kinetic methods to serum creatinine analysis and to compare the effects of glucose and bilirubin on both methods. The study included 100 samples from patients were enrolled to Shree Krishna Hospital Karamsad. The determination of serum creatinine concentrations by enzymatic method on Backman Coulter AU 480 and Modified jaffe’s kinetic method was performed on Siemens Dimension clinical chemistry analyzer (RxL). Results: The correlation coefficient for the two methods in all group (all samples), indicated a very good agreement between Jaffe’s kinetic method and Enzymatic method(r= 0.987, P-value<0.001). The enzymatic method was found to have certain advantages over Jaffe’s kinetic method, and especially lack of interference with substances such as glucose and bilirubin, but it is expensive more than the Jaffe’s method. Conclusion: Enzymatic techniques lead to less variability in serum creatinine measurements than Jaffe techniques and therefore result in more accurate. Therefore the enzymatic techniques are preferably used in clinical diagnosis.
Key words: Creatinine, Enzymatic assay, Jaffe kinetic method, Glucose, Bilirubin.

1. Introduction
Creatinine is one of the kidney function tests(1) Clinical biochemistry laboratories use several methods to estimate the concentrations of serum creatinine. Most common of these methods are Jaffe’s and enzymatic. The Jaffe method is less expensive than the enzymatic method but is also more susceptible to interferences.
analyzed by both Jaffe's kinetic method and the enzymatic method. The serum creatinine determination by Jaffe's method is based on the principle that picric acid in an alkaline medium reacts with creatinine to form a yellow-red complex with the alkaline picrate (10).

The enzymatic method is based on the established determination of hydrogen peroxide after conversion of creatinine with the aid of creatinase and sarcosine oxidase. The liberated hydrogen peroxide reacts with 4 aminophenazone and HTIB to form a quinoneimine chromogen (4, 11). We also estimated serum total bilirubin by colorimetric assay with endpoint Dazio sulfanilic method and plasma glucose by hexokinase method on Siemens Dimension clinical chemistry analyzer. All measurements were performed using the determination of serum creatinine concentrations by the enzymatic method on Backman Coulter AU 480 and Modified jaffe’s kinetic method was performed on Siemens Dimension Clinical Chemistry Analyzer.

**Study design**: cross-sectional study.

**Ethics issues**: This study was approved by the Institutional Ethics Committee (IEC) in Pramukhswami Medical College (PSMC), Karamsad, Sardar Patel University.

**Statistical Analysis**: Descriptive statistics was computed with percentages and proportion. Group comparisons were done by Chi-square test, Pearson correlation and p value, and the mean plus or minus standard deviation (±SD) by SPSS statistical computerized program. References management was done by Endnote X7 program.

### 3. Results:

In this study, the data obtained were divided into four groups. Group I - comprised 17 samples without interfering substances (normal plasma glucose and normal serum total bilirubin); Group II comprised 30 samples with bilirubin (samples with serum total bilirubin > 1.0 mg/dl and normal plasma glucose); Group III comprised 39 samples with plasma glucose (Plasma glucose >126 mg/dl and normal serum of total bilirubin); Group IV comprised 14 samples with interfering substances (increased of bilirubin and glucose).

#### Table 1. The mean differences between the enzymatic and kinetic Jaffe’s methods

<table>
<thead>
<tr>
<th>Groups</th>
<th>Jaffe’s Kinetic Mean</th>
<th>Enzymatic Mean</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Normal</td>
<td>0.87</td>
<td>0.84</td>
<td>0.41</td>
</tr>
<tr>
<td>N</td>
<td>17</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

### Table 1. This table shows a comparison between results of serum creatinine concentration by the enzymatic method and Jaffe’s kinetic method. The mean and SD in Group I (normal) was (0.87± 0.18) in Jaffe’s kinetic compared to enzymatic (0.84± 0.17). In the second group (high bilirubin), was (1.32± 0.66) in Jaffe’s kinetic compared to enzymatic (1.14± 0.58). In Group III was (1.29± 0.88) in Jaffe’s kinetic compared to enzymatic (1.18± 0.81) and Group IV, was (1.32± 0.77) in Jaffe’s kinetic compare to enzymatic (1.24± 0.73). P-values were > 0.05 in all groups, considered to be not quite statistically significant.

#### Table 2. Comparison between enzymatic method and kinetic Jaffe’s method in all groups by Paired Samples Statistic

<table>
<thead>
<tr>
<th>Methods</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinetic Jaffe’s</td>
<td>1.23</td>
<td>100</td>
<td>0.73</td>
</tr>
<tr>
<td>enzymatic</td>
<td>1.11</td>
<td>100</td>
<td>0.67</td>
</tr>
</tbody>
</table>

The mean level was increased in Jaffe’s Kinetic method (1.23± 0.73) more than mean level in enzymatic method (1.11± 0.67) with p-value <0.001.
Table 3. The correlation between enzymatic method and Jaffe’s kinetic method for estimate serum creatinine

<table>
<thead>
<tr>
<th>Methods</th>
<th>N</th>
<th>Correlation</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jaffe’s Kinetic with enzymatic</td>
<td>100</td>
<td>0.987</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

The correlation coefficient for the two methods in all group (all samples), indicated a very good agreement between Jaffe’s kinetic method and Enzymatic method(r= 0.987, P-value<0.001)

4. Discussion:

We try to find if some substances possess any influence on Jaffe’s kinetic method and enzymatic method and try to determine the influence factors. We found the difference between two methods for serum creatinine was not a significant of all group.

High blood glucose can falsely increase in the creatinine results by Jaffe’s kinetic compared to enzymatic methods, which corresponds to the results of the previous study (12-14). However, the use of the Jaffe’s kinetic method resulted in significantly higher creatinine values, compared with those obtained using the enzymatic method.

The enzymatic method of creatinine yields reliable results when the samples take more time to reach the laboratory or delay the blood centrifugation. In a previous study, reported that delays in sample centrifugation caused false increases in measured creatinine by alkaline picrate assay due to the possible interference effect of some metabolites built up in vitro, such as pyruvate or ketones (15).

The correlation coefficient indicated a very good agreement (r= 0.987, P-value<0.001).this confirm by previous study done by Vijaya Marakala and et. at 2012(16).

Although the enzymatic method has certain advantages over the Jaffe’s kinetic method, but it is more expensive.

5. Conclusions:

Enzymatic techniques lead to less variability in serum creatinine measurements than Jaffe techniques and therefore result in more accurate. Therefore the enzymatic techniques are preferably used in clinical diagnosis.

6. References:

5. Gerard S, Khayam-Bashi H. Negative interference with the Ektachem (Kodak) enzymic assay for creatinine by high serum glucose. Clinical chemistry. 1984;30(11):1884-.