Comparison of Jaffé rate assay and enzymatic method for the measurement of creatinine clearance

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To evaluate the relationship between Jaffé rate assay (Jaffé assay) and creatinine amidohydrolase enzymatic method (enzymatic method), we measured serum and urine creatinine concentrations in 100 serum and 100 urine samples by two methods in patients with renal disease. Comparison of Jaffé(X) and enzymatic(Y) measurements of serum and urine creatinine levels revealed a high correlation (r²=0.9991 in serum, r²=0.9995 in urine). The creatinine concentrations in serum and urine determined by Jaffé assay were significantly (p<0.01) higher than those assayed by the enzymatic method. The relationship between Jaffé and enzymatic analyses of creatinine clearance (Ccr) was obtained mathematically using regression lines (Y = 0.977X - 0.199 in serum, Y = 0.999X - 1.872 in urine). Ccr values obtained by both methods were almost the same at high serum creatinine levels. However, Ccr determined by Jaffé assay was much lower than that obtained by the enzymatic method when the serum creatinine concentration was under 2.0 mg/dl. We present here the equation for conversion of clearance values determined by both methods to obtain an accurate evaluation of renal function in many clinical studies.


Key words: creatinine, creatinine clearance, Jaffé rate assay, enzymatic method, equation

Introduction

Ccr is used commonly as an estimate of glomerular filtration rate and is the parameter employed in the evaluation of renal functions. Accurate determination of Ccr requires the accurate measurement of serum and urine creatinine values. There are two routine methods for measuring creatinine; Jaffé assay and the enzymatic method. Jaffé assay is still used commonly in many laboratories, but is not specific for creatinine. Non-creatinine chromogens have been identified in plasma samples, which react positively [1-4]. It is already known that Jaffé assay overestimates serum creatinine values [4-6]. The enzymatic method, which has recently become available for clinical use, is much more accurate and is recommended for creatinine measurement [6-8].

In a collaborative study by many institutions, the values of serum creatinine and Ccr measured by both methods are used for statistical analyses. To increase the reliability of clinical studies, it is important to determine the relationship between Jaffé and enzymatic methods for creatinine measurement and to correct the values according to the methods if necessary. In the present investigation, we developed an equation to convert Ccr values determined by both methods to characterize the correlation of creatinine and Ccr values measured by both methods.

Methods

Creatinine levels in 100 serum and 100 urine samples collected from patients with renal disease was measured by Jaffé assay and the enzymatic method. The Jaffé assay employed was the Creatinine-HR diagnostic kit (Wako, Japan). Enzymatic (creatinine amidohydrolase) method employed was Daiacolor-CRE-S diagnostic kit (Ono, Japan). Measurements were performed on an automated analyzer, Hitachi-7070 (Hitachi, Japan). Serum samples were measured directly. Urine samples were diluted 10-fold with saline before measurement multiplying the result 10-fold. The ranges of serum and urine creatinine that can be determined by Jaffé assay
Comparison of Jaffé rate assay and enzymatic method

Results

1) Relationship between Jaffé assay and the enzymatic method in the measurement of serum and urine creatinine.

Comparison of the Jaffé(X) and enzymatic(Y) measurements of serum creatinine revealed a close correlation, and gave the regression line $Y = 0.977X - 0.199$, $r^2=0.9991$ (Fig. 1A). To evaluate the positive bias of Jaffé measurement, the difference between the serum creatinine values obtained by the two methods (Jaffé – enzymatic) was plotted against the value obtained by Jaffé assay (Fig. 1B). The serum creatinine concentration determined by Jaffé assay was significantly ($P < 0.01$) higher than that found by enzymatic method. The regression line was $(X - Y) = 0.023X + 0.199$ (Fig. 1B), indicating that the positive bias of the Jaffé assay was about 0.2 mg/dl in normal serum creatinine levels and increased more than 0.4 mg/dl when the serum creatinine was about 10.0 mg/dl.

Comparison of Jaffé(X) and enzymatic(Y) measurements on urine creatinine gave the regression line $Y = 0.999X - 1.872$, $r^2=0.9995$ (Fig. 2A). The urine creatinine concentration determined by Jaffé assay was also significantly ($p < 0.01$) higher than that obtained by enzymatic method (Fig. 2B). In contrast to serum creatinine, the positive bias of the Jaffé assay was about 2.0 mg/dl and was relatively constant at all levels of urine creatinine concentrations.

2) Relationship between Ccr values determined by Jaffé assay and the enzymatic method

The enzymatic-to-Jaffé Ccr ratio could be calculated by the regression lines shown in Fig. 1 and Fig. 2, while the Ccr value itself could not be obtained. Although Ccr determination needs the urine volume in addition to serum and urine creatinine concentrations, the ratio between the Ccr values determined by both methods depends only on the creatinine values of serum and urine. When Ccr is measured by Jaffé assay, the positive bias in serum creatinine measurement leads to underestimation of Ccr, whereas the positive bias in urine creatinine measurement leads to overestimation of Ccr. Finally, the accuracy of the Ccr value in Jaffé assay depends on the counterbalance of both effects. At high serum creatinine concentrations, the enzymatic-to-Jaffé Ccr ratio was around 1.0, indicating that Ccr values obtained by both methods were closely correlated. However, the Ccr ratio increased markedly when the serum creatinine concentration was less than 2.0 mg/dl. Ccr by Jaffé assay was much lower than that obtained by the enzymatic method at lower serum creatinine levels (Fig. 3).

Urine creatinine concentration also affected the Ccr ratio, but had little effect compared with serum creatinine concentrations. The difference in the Ccr ratio was less than 10% when the urine creatinine concentration changed from 20 to 340 mg/dl which were the minimum and maximum creatinine concentrations in the present study, respectively (Fig. 3).

3) Conversion (Jaffé→enzymatic) of Ccr value
When serum and urine creatinine as well as Ccr values are known, serum and urine creatinine values expected by the enzymatic method can be calculated from those obtained by Jaffé assay using the regression lines shown in Fig. 1 and Fig. 2. Ccr derived by the enzymatic method is simply calculated from the serum and urine creatinine values obtained by the enzymatic method. When the urine creatinine concentration is not known, an appropriate value for it must be assumed. The urine creatinine concentrations in most patients with decreased Ccr were within 50–100 mg/dl (data not shown). Therefore, the value of 75 mg/dl was used as the urine creatinine concentration in this case. The relationship between the Ccr values obtained by Jaffé assay and the enzymatic method is shown in Fig. 3.

When neither serum nor urine creatinine values are known, some assumptions are required for converting the clearance values obtained by both methods. We assumed the following: (1) Cr{excretion} (mg/day), which is the amount of creatinine excretion determined by Jaffé assay, and (2) V (ml/day), which is the urine volume.

Serum and urine creatinine can be described by the equation below:

\[
SCr_{\text{Jaffé}} = \frac{Cr_{\text{excretion}}}{Ccr_{\text{Jaffé}}}/14.4
\]

\[
UCr_{\text{Jaffé}} = 100 \cdot \frac{Cr_{\text{excretion}}}{V}
\]

where Ccr{Jaffé} (ml/min) is Ccr obtained by Jaffé assay; SCr{Jaffé} and UCr{Jaffé} (mg/dl) are serum and urine creatinine concentrations obtained by Jaffé assay respectively.

Serum and urine creatinine values expected by the enzymatic method can be obtained from those determined by Jaffé assay using the regression line below:

\[
SCr_{\text{enzyme}} = a \cdot SCr_{\text{Jaffé}} + b \quad (a = 0.977, \quad b = -0.199 \text{ from Fig. 1})
\]

\[
UCr_{\text{enzyme}} = a' \cdot UCr_{\text{Jaffé}} + b' \quad (a' = 0.999, \quad b' = -1.872 \text{ from Fig. 2})
\]

where SCr{enzyme} and UCr{enzyme} (mg/dl) are serum and urine creatinine values obtained by the enzymatic method, respectively.

Ccr obtained by the enzymatic method can be obtained by the equation below:

\[
Ccr_{\text{enzyme}} = \frac{UCr_{\text{enzyme}} \cdot V}{SCr_{\text{enzyme}}} / 1440
\]

\[
Ccr_{\text{enzyme}} = \frac{(a' \cdot (100 \cdot Cr_{\text{excretion}}/V) + b') \cdot V/(a \cdot (Cr_{\text{excretion}}/Ccr_{\text{Jaffé}})/14.4) + b'}{1440}
\]

\[
Ccr_{\text{enzyme}} = \frac{(99.9 \cdot Cr_{\text{excretion}} - 1.872 \cdot V)/(97.7 \cdot Cr_{\text{excretion}}/Ccr_{\text{Jaffé}} - 286.56)}{1440}
\]

where Ccr{enzyme} is Ccr obtained by the enzymatic method.

Substitution of 500, 1000 and 1500 mg/day for Cr{excretion}, and 1500 ml for V shows the relationship between Ccr values obtained by Jaffé assay and the enzymatic method (Fig. 4). At normal creatinine clearance levels, Ccr determined by Jaffé assay was much lower than that obtained by the enzymatic method. This...
underestimation of Ccr by Jaffé assay was more critical when the urine creatinine excretion was low (500 mg/day). However, the difference between Ccr values obtained by Jaffé assay and the enzymatic method reduced as the Ccr value decreased.

**Discussion**

Comparison of Jaffé and enzymatic creatinine measurements on the same serum and urine samples confirmed that the values obtained by Jaffé assay correlated to, but were significantly higher than those obtained by the enzymatic method. It has been known that serum and urine contain non-creatinine chromogens which react positively in Jaffé reaction, contributing to the difference between the creatinine values determined by two methods [4]. We demonstrated that the positive bias in serum creatinine values in Jaffé assay is about 0.2 mg/dl in normal creatinine levels and increased up to 0.5 mg/dl as serum creatinine increased (Fig. 1).

Overestimation of serum creatinine by Jaffé assay leads to underestimation of Ccr. The enzymatic to Jaffé clearance ratio increased up to 2.0 at low serum creatinine concentrations (Fig. 3). Ccr obtained by Jaffé assay was only half of the value obtained by the enzymatic method when the serum creatinine concentration was 0.4 mg/dl. However, the enzymatic to Jaffé clearance ratio became almost 1.0 when the serum creatinine concentration was more than 2.0 mg/dl, indicating a much better correlation between the two methods at high serum creatinine levels. The magnitude of underestimation of Ccr by Jaffé assay depended on the proportion of the positive bias against the total creatinine concentration in the serum samples. The regression line in Fig. 1 showed that the proportion of the bias was more significant at lower creatinine concentrations and resulted in a poor performance of Jaffé assay in Ccr measurement at low creatinine levels.

Urine creatinine concentration also affected the relation between Ccr values obtained by Jaffé assay and the enzymatic method, but had little effect compared with the serum creatinine concentrations. The proportion of the bias against the total urine creatinine concentration was less than 10% at any creatinine level. Therefore, the difference in the Ccr ratio was less than 10% when the urine creatinine concentration changed from 20 mg/dl to 340 mg/dl, which were the minimum and maximum creatinine concentrations in the present study, respectively. Urine creatinine concentrations may be within a more narrow range in most patients, particularly in patients with renal failure because of their low urine concentrative ability. Consequently, urine creatinine concentrations are not important for the conversion of Ccr values obtained by the two methods.

The underestimation of Ccr by Jaffé assay was more significant when daily creatinine excretion decreased from 1500 to 500 mg/day (Fig. 4). According to the formula for Ccr calculation, lower excretion of creatinine with an identical Ccr value gave a lower serum creatinine concentration, that lead to underestimation of Ccr by Jaffé assay. This result suggested that underestimation of Ccr by Jaffé assay was more prominent in patients with a smaller amount of muscles, such as children, women and aged persons. When the amount of creatinine excretion is not known, an estimated value in individual patients may be useful for more accurate conversion of Ccr. Several equations have been reported for estimating the amount of creatinine excretion from body weight, age, and sex [9, 10].

We concluded that underestimation of Ccr by Jaffé assay may be critical in patients with serum creatinine values under 2.0 mg/dl. The equation that we have presented in this study, should be useful for the evaluation of Ccr by Jaffé assay in these patients.

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**References**