Creatinine determination in peritoneal dialysis: what method should be used?

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Abstract The accuracy of methods for measurement of creatinine in plasma, urine and dialysate is of great importance in continuous ambulatory peritoneal dialysis (CAPD) patients, to assess the adequacy of CAPD (creatinine clearance) and to monitor the nutritional status (creatinine kinetic lean body mass). The methods most widely employed for creatinine determination are Jaffe’s reaction and the enzymatic method, however these techniques may suffer from glucose interference, particularly for dialysate. We compared creatinine values obtained by Jaffe’s reaction, the enzymatic method and high pressure liquid chromatography (HPLC) for three creatinine calibration curves prepared in three dialysis solutions with various concentrations of glucose and for plasma, urine and dialysate of 40 CAPD patients. High values of intercept of creatinine calibration curves were observed only with Jaffe’s reaction and the enzymatic method in dialysis solutions. In plasma, urine and dialysate, creatinine values obtained by HPLC were always found to be lower than those measured by the other two methods. Concerning creatinine measurement in plasma and urine, Jaffe’s reaction and the enzymatic method appeared equivalent. However it must be noted that, in dialysates, the enzymatic method may have glucose interference, and the use of a correcting factor for glucose with Jaffe’s reaction is convenient. Nevertheless HPLC remains a method of reference. It is concluded that, for the CAPD patient, follow-up by creatinine kinetic lean body mass or creatinine clearance is possible provided that the same creatinine assay method is used in all biological fluids.

Key words: continuous ambulatory peritoneal dialysis; creatinine; enzymatic assay; high-pressure liquid chromatography; Jaffe’s reaction

Introduction

To assess the adequacy of continuous ambulatory peritoneal dialysis (CAPD), the main indicator has always been clinical outcomes, such as patient survival, hospitalization frequency, transfusion rate and patient symptoms, however these parameters are difficult to quantify [1,2]. Urea and creatinine kinetic modelling are now accepted for defining the adequacy of CAPD [3], even though the optimal biochemical targets for urea and creatinine clearance are still debated. Nutritional status of CAPD patients is a strong parameter of technical outcome, and is frequently impaired by the loss of appetite due to glucose absorption, dialysate protein losses, and insufficient dose of dialysis [4]. Monitoring the nutritional status of CAPD patients requires a simple method for measuring body composition. A simple method lean body mass (LBM) estimation by creatinine kinetics has recently been proposed and validated in dialysis patients. It is based on the principle that creatinine production is proportional to LBM and that, in a steady state, creatinine production is equal to the sum of creatinine excretion (urinary and dialytic) and metabolic degradation [5].

Therefore, the accuracy of methods for measurement of creatinine in plasma, urine and especially in dialysate is of importance. The methods for creatinine determination most widely employed for assays in clinical laboratories are Jaffe alkaline-picrate, a colorimetric method, and more recently enzymatic procedures. The major disadvantage of both methods is the positive interference by endogenous substances [6–8]. While high concentrations of bilirubin [9,10] and glucose [11,12] are the main interferences with the Jaffe’s method, it should be noted that, to a lesser extent, high concentrations of glucose, ammonia or amino acids interfere significantly with the creatinine enzymatic assay [13,14]. Several methods were developed to measure ‘true creatinine’, particularly high pressure liquid chromatography (HPLC) [15,16], which now represents a reference method.

The aim of this work was to determine the influence of the creatinine assay method on creatinine clearances...
and LBM estimation from creatinine kinetics. Jaffe’s reaction, enzymatic assay and HPLC were compared for this purpose.

**Subjects and methods**

**Sampling**

Heparinized plasma from 40 patients on CAPD was collected. Dialysate from the last 24 h was mixed, and urine was collected during the same period. Two of 40 patients had no residual renal function. The samples were stored at −18°C until analysis. In order to evaluate the influence of glucose concentration in dialysis solutions, and the influence of the method on creatinine values, three series of dilutions of creatinine were prepared between 0 and 1.77 mmol/l in three dialysis solutions containing 1.36%, 2.27% and 3.86% glucose, respectively.

**Analytical methods**

Creatinine was measured in plasma, urine and dialysate by three different methods: (1) an automated Jaffe’s reaction preceded by dialysis on an autoanalyser (Technicon AA II-11); (2) an enzymatic method using the CREA Kit Kodak Ektachem (Eastman Kodak Co, Rochester, NY); and (3) a reversed-phase HPLC with ultraviolet detection at a wavelength of 220 nm, on a Kontron automated system (Kontron, Zürich, Switzerland). The coefficients of variation of the three methods were never found higher than 6.9%, 6.3% and 3.1%, respectively, for HPLC, Jaffe’s reaction and enzymatic method, whatever the biological fluids. Glucose was determined in dialysate by the enzymatic glucose oxidase method using Glucose Enzymatique PAP (BioMerieux, Marcy l’Etoile, France).

**CAPD adequacy parameters**

Several parameters used to estimate CAPD adequacy were calculated, i.e., renal, peritoneal and total creatinine clearances (ml/min) and creatinine generation rate as a convenient index of renal function. The samples were stored at −18°C until analysis. In order to evaluate the influence of glucose concentration in dialysis solutions, and the influence of the method on creatinine values, three series of dilutions of creatinine were prepared between 0 and 1.77 mmol/l in three dialysis solutions containing 1.36%, 2.27% and 3.86% glucose, respectively.

**Results**

The influence of glucose concentration in dialysate is shown in Table 1. Slopes and intercept values (mmol/l) were obtained from a linear regression test performed to compare calibration curves of creatinine (not corrected for glucose interference) determined by the three methods, in three dialysis solutions with various concentrations of glucose. For the enzymatic method, we observed a decrease of the slope with increasing glucose concentrations. For the enzymatic method, we observed a decrease of the slope with increasing glucose concentrations (from 0.994 for 1.36% to 0.884 for 3.86%). An increase of intercept values was associated with the increase of glucose concentration for both Jaffe’s reaction and the enzymatic method, while low intercept values were observed with HPLC (±0.004 mmol/l).

In Figs 1, 2 and 3, the linear regression between the creatinine values obtained in plasma, urine and dialysate, respectively, by the three methods of creatinine determination show good correlations; coefficients were always higher than $R^2=0.926$ ($P<0.001$), except for the relationship between the enzymatic method and HPLC, in urine, where $R^2=0.826$ ($P<0.001$). Nevertheless, as shown in Table 2, creatinine values obtained by Jaffe’s reaction and the enzymatic assay were always greater than those measured by HPLC. This overestimation was significant ($P<0.05$) in plasma for Jaffe’s reaction, while in dialysate and urine no significant difference was found. As shown in Figs 1–3, the linear regression between the difference of

**Table 1.** Slopes and intercept values (mmol/l) of calibration curves of creatinine in dialysis solutions with three different concentrations of glucose (%), determined by HPLC, Jaffe’s reaction and the enzymatic method.

<table>
<thead>
<tr>
<th>Glucose concentration (%)</th>
<th>HPLC</th>
<th>Jaffe’s reaction</th>
<th>Enzymatic method</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Slope</td>
<td>Intercept value</td>
<td>Slope</td>
</tr>
<tr>
<td>1.36</td>
<td>0.990</td>
<td>−0.003</td>
<td>1.07</td>
</tr>
<tr>
<td>2.27</td>
<td>0.978</td>
<td>−0.002</td>
<td>1.04</td>
</tr>
<tr>
<td>3.86</td>
<td>0.971</td>
<td>0.004</td>
<td>1.05</td>
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</table>
individual values of creatinine, in all biological fluids, obtained by two of three methods and the mean of the two values showed a significant correlation for plasma creatinine concentrations only when Jaffe’s method was compared with HPLC ($R^2 = 0.515, P < 0.05$) or the enzymatic assay ($R^2 = 0.284, P < 0.05$).

Parameters calculated to estimate CAPD adequacy are shown in Table 2. Whatever the method, renal, peritoneal and total clearances were not significantly different. The lowest values of LBM were always found with the HPLC method, however the differences became significant only for LBM measured by Jaffe’s reaction. No differences between Jaffe’s reaction and the enzymatic method could be disclosed in any case, as well as for creatinine values or kinetic parameters.

Discussion

This work aimed to assess the influence of choice of creatinine assay method on the determination of creatinine in plasma, urine and dialysate, more or less impaired by high glucose concentrations, and its consequences on creatinine clearance and creatinine kinetics LBM, classically used in CAPD patients follow-up.

As has been described [11,12], high glucose concentrations in dialysis solutions constitute the major interference in creatinine determination by Jaffe’s reaction. Although these results demonstrate interference of glucose with enzymatic creatinine determination, some authors have denied this and advise its use rather than the Jaffe’s reaction in dialysate [11]. Effectively, high intercept values of calibration curves of creatinine in dialysis solutions showed that both methods, Jaffe’s reaction and the enzymatic method, gave false concentrations in dialysates. Moreover, for the enzymatic assay the decrease of slopes associated with high glucose concentrations could lead to an underestimation of high creatinine values. The low intercept values in dialysis solutions obtained by HPLC showed the lack of glucose interference with creatinine determination, and therefore this latter technique appears a more accurate and reliable method for creatinine measurement in peritoneal dialysates. However, creatinine
values estimated in patient dialysate with corrected Jaffe’s reaction and the enzymatic method did not show any significant difference from the HPLC technique. The use of a correction coefficient for Jaffe’s method appears convenient [1,18,19].

The comparison between the three methods in plasma, urine or dialysate indicated a linear correlation; these findings are in accordance with the literature [20]. The values of plasma creatinine determined by HPLC were always lower than those obtained by other methods. The only significant difference was observed in plasma between Jaffe’s method and HPLC. In

Table 2. Creatinine values (mean±SD, n=40) determined by HPLC, Jaffe’s reaction and the enzymatic method in plasma, urine and dialysate; parameters of CAPD adequacy (creatinine clearances) and creatinine kinetic lean body mass (LBM). (Jaffe’s reaction vs HPLC: *P<0.05)

<table>
<thead>
<tr>
<th></th>
<th>HPLC</th>
<th>Jaffe’s reaction</th>
<th>Enzymatic method</th>
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</thead>
<tbody>
<tr>
<td>Plasma (mmol/l)</td>
<td>0.76±0.28</td>
<td>0.94±0.33*</td>
<td>0.81±0.29</td>
</tr>
<tr>
<td>Urine (mmol/l)</td>
<td>2.80±1.44</td>
<td>3.64±1.70</td>
<td>3.32±1.58</td>
</tr>
<tr>
<td>Dialysate (mmol/l)</td>
<td>0.49±0.26</td>
<td>0.53±0.27</td>
<td>0.51±0.26</td>
</tr>
<tr>
<td>Renal clearance (ml/min)</td>
<td>4.24±3.85</td>
<td>4.43±3.74</td>
<td>4.63±3.71</td>
</tr>
<tr>
<td>Peritoneal clearance (ml/min)</td>
<td>4.38±1.21</td>
<td>3.80±0.95</td>
<td>4.21±1.01</td>
</tr>
<tr>
<td>Total clearance (ml/min)</td>
<td>8.62±3.27</td>
<td>8.22±3.17</td>
<td>8.85±3.16</td>
</tr>
<tr>
<td>LBM (kg)</td>
<td>41.06±6.95</td>
<td>47.04±7.61*</td>
<td>43.87±7.02</td>
</tr>
<tr>
<td>LBM_u (kg)</td>
<td>11.92±9.11</td>
<td>15.44±10.76</td>
<td>13.85±9.08</td>
</tr>
<tr>
<td>LBM_d (kg)</td>
<td>16.25±8.18</td>
<td>17.45±8.56</td>
<td>16.78±8.50</td>
</tr>
<tr>
<td>LBM_e (kg)</td>
<td>5.51±2.27</td>
<td>6.78±2.65</td>
<td>5.86±2.37</td>
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</table>
addition, the significant correlation between the difference and the mean of creatinine values obtained in plasma showed that the difference was increasing with the values of creatinine. The dilutions of plasma required for high concentrations of creatinine in CAPD patients could explain the increase of the difference observed as a function of creatinine concentration. On the other hand, we assume that a problem of calibration standard may occur: for HPLC, the calibration standard was an aqueous solution of creatinine for all biological fluids, while proteins were included in the other methods. No significant difference was found in urine and dialysate, in spite of the non-convenient protein calibration standard for the enzymatic method.

The choice of creatinine assay in biological fluids and dialysate is of importance in the calculation of CAPD adequacy parameters and the follow-up of CAPD patients' nutritional status by LBM [5,21]. Considering creatinine clearance, no significant differences were observed with the three methods, even though Jaffe plasma creatinine was significantly higher than HPLC. We assume that calculating a clearance with the same assay method for urine, plasma and dialysate reduced the observed differences for creatinine values in each biological fluid. This is confirmed by the significantly higher Jaffe LBM, due to the importance of the plasma creatinine value in the LBM formula [5]. Creatinine kinetics LBM should be validated in each centre with a reference LBM determination; nevertheless follow-up of LBM should be available for CAPD patients provided that the same creatinine assay method is used.

In conclusion, concerning creatinine measurement in plasma and urine, Jaffe's reaction and the enzymatic method appeared equivalent. However, when considering the influence of glucose in dialysate, it must be noted that the enzymatic method is not totally devoid of interference, and that Jaffe's reaction associated with a correction factor for glucose interference is an alternative to the enzymatic technique. Nevertheless, it appears that HPLC remains a method of reference. Considering the clinical follow-up of one
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CAPD patient, in terms of creatinine clearance or creatinine kinetics LBM, we recommend the use of a single creatinine assay method for all biological fluids.

References


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