The Glucose Interference in Creatinine Measurement Using an Enzymatic Method: Effect of Creatinine Concentrations

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Objective: *To evaluate the influence of glucose and creatinine concentrations on the determination of creatinine by Jaffe picrate reaction and specific enzymatic assay.*
Material and Method: *Unused dialysate with 4.25% dextrose was diluted to obtain seven glucose concentrations. Two series of dialysate were spiked with creatinine to yield concentrations of 5 and 10 mg/dl. Creatinine measurements were obtained by Jaffe method and enzymatic assay.*
Results: *In unused dialysate solution with glucose concentrations from 559 to 4,250 mg/dl, the creatinine values obtained by the Jaffe method were higher than the enzymatic assay (0.31 $\pm$ 0.20 vs. 0.08 $\pm$ 0.01 mg/dl, $p < 0.05$). The correlation coefficient between glucose and creatinine from the Jaffe method were 0.98 ($p < 0.001$) but showed no correlation with creatinine measured with the enzymatic assay. On the other hand, the mean values of creatinine in dialysate with creatinine concentrations of 5 and 10 mg/dl derived by Jaffe method were lower than enzymatic assay (5.74 $\pm$ 0.12 vs. 6.16 $\pm$ 0.36 mg/dl and 11.56 $\pm$ 0.17 vs. 12.69 $\pm$ 0.66 mg/dl, respectively). At creatinine concentration of 10 mg/dl, the correlation between glucose concentration and creatinine from enzymatic assay was significant. In contrast, at creatinine concentration of 5 mg/dl, the correlations obtained from both methods were significant.*
Conclusion: *The patterns of glucose interference with creatinine obtained from Jaffe method and enzymatic assay were quite different. The magnitude of interference with enzymatic assay was greater at a higher creatinine concentration. Therefore, the enzymatic assay might not be appropriate for creatinine measurement in patients using dialysate with dextrose 4.25% and membrane characteristic of high solute transporter.*

Keywords: *Creatinine measurement, Dialysate, Jaffe alkaline picrate reaction, Specific enzymatic assay*

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In peritoneal dialysis (PD), peritoneal membrane serves as an artificial dialysis membrane to remove waste product and maintain fluid-electrolyte homeostasis. Peritoneal equilibration test (PET) is generally accepted as the gold standard to determine the peritoneal membrane transport characteristic. The dialysis prescription such as dwelling-time in peritoneal cavity, the concentration of the dialysate solution used and the dose of dialysis performed daily, is based upon this characteristic. Determination of this characteristic by PET depens on the ratio between the creatinine concentration in the peritoneal dialysate at specific hours of dwell-time and the value of plasma creatinine at certain hour[1]. Erroneous results in creatinine measurement can result in misleading in dialysis treatment. Therefore, the accuracy of methods for creatinine measurement in plasma and dialysate is important.

There are several methods in measuring creatinine, including Jaffe alkaline-picrate reaction, enzymatic assay, high-performance liquid chromatography (HPLC), and isotope dilution mass spectrometry (IDMS). Both HPLC and IDMS are more reliable,

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representing as the reference methods[2]. Unfortunately, both are available only in very few laboratories worldwide. Jaffe alkaline-picrate reaction is the most widely used method of creatinine measurement in clinical biological fluid since the test is inexpensive and easy to perform. The major disadvantage of Jaffe reaction is the interference of creatinine measurement by some certain substances, including high glucose[2]. The enzymatic assay is theoretically more specific, but less common in use for creatinine measurement. While very high glucose concentration definitively interferes with the Jaffe method on creatinine measurement, the influence of high glucose with the enzymatic assay is equivocal[3,4]. Nevertheless, erroneous results in creatinine determination can occur if the glucose interference is not taken into consideration.

The aim of the present study was to evaluate the influence of glucose and creatin in econcentrations on the determination of creatinine in both the Jaffe method and enzymatic assay.

Material and Method

In order to evaluate the influence of glucose on creatinine determination, the authors prepared 7 dilutions of a fresh commercial dialysate solution initially containing 4.25% (w/v) dextrose (PD 2, Baxter Healthcare Corporation Woodland, Singapore). Dilutions were performed to obtain the glucose concentrations between 525-4,250 mg%. Other series of dialysate solutions with seven different glucose concentrations were spiked with standard creatinine powder (BDH Chemical, Ltd.) to yield creatinine concentrations of 5 and 10 mg%.

The authors measured creatinine with the Konelab 60 analyzer (Thermo Scientific, Finland) employing Jaffe alkaline picrate reaction and the automated Vitros 5,600 (Johnson & Johnson Company, USA) using creatinine specific enzyme and creatine aminohydrolase. Glucose was assessed by an enzymatic glucose oxidase method on the Konelab 60 analyzer. The analyzer settings for these measurements were set according to the manufacturer's guideline. Specimens were determined in duplicate.

Statistical analysis

All data were expressed as mean $\pm$ standard deviation unless otherwise stated. The comparison between creatinine concentrations derived from Jaffe reaction and enzymatic assay was performed with paired samples t-test. The association between creatinine and glucose concentrations was analyzed by Pearson or Spearman rank correlation coefficient. The level of statistical significance was set at $p < 0.05$. The statistical software program R for Windows, version 2.12.0 were used for statistical analysis.

Results

In an unused dialysate solution with varying glucose concentrations from 595 to 4,250 mg%, the creatinine values found with the Jaffe method were different from those with the enzymatic assay (Table 1). The mean values of creatinine concentration obtained from the Jaffe reaction were significantly higher than the enzymatic assay (p-value < 0.05). The correlation between glucose and creatinine concentrations from the Jaffe method was significant (Table 1).

In fresh dialysate solution spiked with standard creatinine at fixed creatinine concentrations of 5 and 10 mg%, the mean values of creatinine concentration obtained with the Jaffe method were

Table 1. The mean creatinine and correlation between glucose and creatinine measured with Jaffe method and enzymatic assay

Creatinine in standard solution	Method	Mean creatinine (SD) in dialysate	r (p-value)
0	Jaffe	0.31 $\pm$ 0.20	0.98*
0	Enzymatic	0.08 $\pm$ 0.01**	0.087
5.7	Jaffe	5.74 $\pm$ 0.12	0.77*
5.7	Enzymatic	6.16 $\pm$ 0.36**	0.88*
11.4	Jaffe	11.56 $\pm$ 0.17	0.19
11.4	Enzymatic	12.69 $\pm$ 0.66**	0.81*

* $p < 0.05$, correlation between glucose and creatinine concentration
** $p < 0.05$, comparsion between creatinine measured by Jaffe and enzymatic method

lower than the enzymatic assay (Table 1). At creatinine concentration of 10 mg%, the correlation between glucose concentration and creatinine derived from enzymatic assay was significant. In contrast, at creatinine concentration of 5 mg%, the correlations obtained from both methods were significant (Table 1).

Discussion

The present study found that high glucose interfered with creatinine determination both on the Jaffe method and the enzymatic assay. However, the patterns of interference were quite different. In contrast to the effect of glucose on creatinine measurement on the Jaffe method, the magnitude of interference on the enzymatic assay was greatly significant at higher creatinine concentration.

The authors found significant discrepancies between creatinine performed by the Jaffe method and the enzymatic assay in unused dialysate solutions. Indeed, the interference of glucose on creatinine measurement using the Jaffe method has been widely recognized. The influence of glucose can be explained by the knowledge that glucose could slowly reduce the alkaline picric acid to picrate to form an artificial colored complex[5]. This effect is insignificant in most situations. However, in dialysate solution containing very high glucose, this interference is considerably important. The present study demonstrated that the interference did not only depend on glucose but also on creatinine. The interference of high glucose concentration in the Jaffe method was not significant with high creatinine concentration. At any glucose concentrations, the higher the creatinine concentration, the less interference from glucose, and the more accurate with creatinine determination. This might be due to the competition of creatinine and glucose for the limited amount of picrate.

The interference of creatinine measurement with the enzymatic assay is still inconclusive. Several authors observed that the results from the enzymatic assay were lower than the Jaffe method[6]. An earlier study reported by Larpent and Verger[3] showed that creatinine measurement in dialysate solution with enzymatic method was not influenced by high glucose. They suggested that the enzymatic assay was an appropriate method for creatinine measurement. According to the present study, it was the case only when creatinine level was measured in fresh unused dialysate, which is free of creatinine. At creatinine concentrations of 5 and 10 mg%, the results from the enzymatic assay were higher than the Jaffe method. However, this interference was associated with glucose concentration. This was in agreement with the positive interference reported by Mak et al[4]. The explanation of this interference is uncertain but it may relate to the detection system and/or the enzyme[7].

Therefore, creatinine measurements obtained from the Jaffe method and the enzymatic assay should be interpreted with caution in dialysate solutions containing high glucose concentration. Besides the glucose concentration for interference, it is important to consider both the method of measurement and creatinine concentration and to adjust the creatinine level in dialysate solution. Without correction, the result of the PET obtained from such value of creatinine would be inaccurate and cause misleading of the dialysis treatment. The following correction equation to account for the glucose interference was proposed: corrected creatinine = measured creatinine (correction factor x glucose concentration)[8]. The correction factor was developed from creatinine determination in unused dialysate divided by dialysate glucose. As the pattern of glucose interference varies with the method, the Jaffe method and the enzymatic assay, and also the creatinine concentration, the common correction formula for all the systems evaluated cannot be drawn. Furthermore, the enzymatic assay was unreliable to measure creatinine at high glucose and creatinine concentrations. The authors suggested that the enzymatic assay should not be used for creatinine measurement without any correction in the PET test, particularly at the 2nd and 4th time of dwelling. Alternatively, either the method for creatinine measurement, unaffected by glucose or the Jaffe method using correction factor derived from the fresh dialysate creatinine and glucose should be considered.

In conclusion, when one considers the influence of glucose in dialysate, it must be noted that the enzymatic method is not totally devoid of interference. However, the pattern of interference was quite different. In contrast to the effect of glucose on creatinine measurement by the Jaffe method, the magnitude of interference was actually higher at high levels of creatinine concentration.

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Potential conflicts of interest

None.

References

1. Twardowski ZJ, Nolph KD, Khanna R, Prowant BF, Ryan LP, Moore HL, et al. Peritoneal equilibration test. Perit Dial Bull 1987; 7: 138-47.
2. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, Greene T, et al. Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin Chem 2006; 52: 5-18.
3. Larpent L, Verger C. The need for using an enzymatic colorimetric assay in creatinine determination of peritoneal dialysis solutions. Perit Dial Int 1990; 10: 89-92.
4. Mak TW, Cheung CK, Cheung CM, Leung CB, Lam CW, Lai KN. Interference of creatinine measurement in CAPD fluid is dependent on glucose and creatinine concentrations. Nephrol Dial Transplant 1997; 12: 184-6.
5. Narayanan S, Appleton HD. Creatinine: a review. Clin Chem 1980; 26: 1119-26.
6. Perakis N, Wolff CM. Kinetic approach for the enzymic determination of creatinine. Clin Chem 1984; 30: 1792-6.
7. Gerard S, Khayam-Bashi H. Negative interference with the Ektachem (Kodak) enzymic assay for creatinine by high serum glucose. Clin Chem 1984; 30: 1884.
8. Tam P, Sheldrake P, Ng A, Oreopoulos DG, Sloand JA. Peritoneal equilibration testing: correcting the correction factor. Perit Dial Int 2009; 29: 352-5.

การรบกวนการวัดคริแอทินีนด้วยอิทธิพลของน้ำตาลกลูโคส: บทบาทความเข้มข้นของคริแอทินีน

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วัตถุประสงค์*: เพื่อประเมินบทบาทของความเข้มข้นน้ำตาล และคริแอทินีนต่อการรบกวนการวัดคริแอทินีนทั้งการวัดด้วยเจฟฟี่และเอนไซม์*

วัสดุและวิธีการ*: เจือจางน้ำยาล้างไตชนิดน้ำตาลเด็กซ์โตส 4.25% ที่ไม่เคยใช้ให้ได้ความเข้มข้นของน้ำตาลรวม 7 ระดับ ผสมผงคริแอทินีนในน้ำยาให้ได้ความเข้มข้น 5 และ 10 มิลลิกรัม/เดซิลิตร ร่วมกับเจือจางให้ได้น้ำตาลความเข้มข้น 7 ระดับ เช่นกัน หลังจากนั้นนำน้ำยาทั้งสองกลุ่มนี้ วัดระดับคริแอทินีนด้วยวิธีเจฟฟี่และเอนไซม์*

ผลการศึกษา*: ระดับคริแอทินีนในน้ำยาที่ไม่เคยใช้และมีความเข้มข้นกลูโคสตั้งแต่ 595-4,250 มิลลิกรัม/เดซิลิตร ที่วัดด้วยวิธีทั้งสองมีค่าแตกต่างกันโดยวิธีเจฟฟี่วัดได้ 0.31 $\pm$ 0.20 มิลลิกรัม/เดซิลิตร ส่วนวิธีเอนไซม์วัดได้ 0.08 $\pm$ 0.01 มิลลิกรัม/เดซิลิตร ($p < 0.05$) ระดับคริแอทินินที่วัดด้วยวิธีเจฟฟี่มีความสัมพันธ์กับน้ำตาล ($r = 0.98$, $p < 0.001$) แต่ไม่พบความสัมพันธ์เมื่อวัดด้วย วิธีเอนไซม์ ส่วนน้ำยาที่มีคริแอทินิน 5 และ 10 มิลลิกรัม/เดซิลิตร พบว่าคริแอทินินจากการวัดด้วยวิธีทั้งสองแตกต่างกันแต่ได้ผลตรงข้าม คือ วิธีเจฟฟี่วัดได้ต่ำกว่าวิธีเอนไซม์ (5.74 $\pm$ 0.12 vs. 6.16 $\pm$ 0.36 มิลลิกรัม/เดซิลิตรและ 11.56 $\pm$ 0.17 vs. 12.69 $\pm$ 0.66 มิลลิกรัม/เดซิลิตร ตามลำดับ) ในน้ำยาที่มีความเข้มข้นของคริแอทินิน 10 มิลลิกรัม/เดซิลิตร พบความสัมพันธ์ระหว่างน้ำตาลกับคริแอทินินที่วัดด้วยวิธีเอนไซม์เท่านั้น ส่วนน้ำยาที่มีความเข้มข้นของคริอะตินีน 5 มิลลิกรัม/เดซิลิตร พบความสัมพันธ์ดังกล่าวทั้งสองวิธี*

สรุป*: น้ำตาลที่สูงในน้ำยาล้างไตรบกวนการวัดคริแอทินินด้วยวิธีเจฟฟีและเอนไซม์ แต่มีรูปแบบแตกต่างกัน โดยการวัดวิธีเอนไซม์จะถูกรบกวนในน้ำยาที่มีคริแอทินินสูง ดังนั้นการวัดคริแอทินินด้วยวิธีเอนไซม์อาจไม่เหมาะสมในรายที่ใช้น้ำยาที่มีความเข้มข้นของเด็กโตส 4.25% และมีเนื้อบุช่องท้องชนิดแลกเปลี่ยนสารเร็ว*