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Glucose interference in serum and urine samples with various creatinine concentrations measured by the Jaffe kinetic method

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ABSTRACT

Background

The effect of glucose interference on creatinine measurement by Jaffe kinetic method differs between serum and urine specimens. We investigated the effects of creatinine concentration and specimen dilution on glucose interference with urine creatinine measurement.

Methods

Leftover serum and urine specimens were collected and stored at -20℃ until study. Serum specimens were mixed to make 5 glucose concentrations ranging from <5.6 to 27.8 mmol/L, each group consisting of 5 levels of creatinine concentration ranging from <45 to 354 µmol/L. Urine specimens were divided into 5 groups of creatinine concentration ranging

from <1,769 to >7956 µmol/L, each sample was spiked with glucose powder to produce 5 aliquots with glucose concentrations ranging from 0 to 666 mmol/L. Urine samples were automatically diluted 1:20 before analysis. Percent interference of creatinine measurement by Jaffe kinetic method was calculated using enzymatic method as the reference.

Results

A total of 148 serum samples and 335 urine samples were analyzed. In serum, glucose interference with Jaffe creatinine measurement was found if creatinine concentrations were 177 µmol/L or less, corresponding to 3,540 µmol/L or less in urine specimens prior to 1:20 dilution. The degree of interference was greater when glucose concentration was higher or creatinine concentration was lower.

Conclusions

When creatinine concentration and specimen dilution were considered, the effects of glucose interference on Jaffe creatinine measurement were similar in serum and urine specimens, and was found when creatinine concentrations in serum or diluted urine were 177 µmol/L or less.

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INTRODUCTION

Creatinine measurement either in serum or urine is one of the most common routine laboratory tests. Generally, serum creatinine is used as an indicator of glomerular filtration rate while urine creatinine is used for hydration correction in spot urine sample. Even though the enzymatic method is accepted as an accurate method for creatinine measurement and deals effectively with most interfering substances, Jaffe kinetic is still the commonly used method for determining creatinine because of its simplicity and low cost. The Jaffe reaction is a colorimetric method and it is well known that non-specific chromogens, especially glucose, falsely increase the results. However, most of the data of glucose interference with Jaffe creatinine measurement were from the studies using serum or peritoneal dialysate [1-9]. There was only one study that investigated the interfering effect of glucose on creatinine measurement in urine and found that glucose did not significantly influence the measurement of urine creatinine by the Jaffe kinetic method even if the concentration of glucose was extremely high at 320 mmol/L [10]. As normal urine creatinine concentration is much higher than serum creatinine concentration, urine samples must be diluted by 20-25 folds before analysis. This dilution process also lowers the concentration of glucose in the urine and might abolish the interference effect of glucose. The discrepant results between the studies using serum and urine specimens could be explained by the difference in creatinine concentration and the dilution process of urine sample prior to measurement.

In the era of sodium-glucose cotransporter 2 (SGLT2) inhibitors, urine glucose excretion may be found at more than 100 g/day [11]. Theoretically, these extremely high glucose concentrations may interfere with urine creatinine assay by the Jaffe kinetic method even though the urine specimen is diluted 20 times prior to measurement. To prove this concept, we studied the interference of glucose on Jaffe creatinine assay in a broad range of glucose and creatinine concentrations, and in both serum and urine specimens.

MATERIALS AND METHODS

This cross sectional study was conducted in the biochemical laboratory of Songklanagarind Hospital during the year 2021. Leftover serum and urine specimens were collected and prepared in different concentrations of glucose

and creatinine. The effects of glucose interference with creatinine measurement by the Jaffe kinetic method were studied in both serum and urine samples. Since the enzymatic method is an accurate method for creatinine measurement without glucose interference, it was used as a reference method. Percent interference (%) of creatinine measurement by the Jaffe kinetic method was calculated as 100 x (creatinine concentration by the Jaffe kinetic method – creatinine concentration by the enzymatic method) ÷ creatinine concentration by the enzymatic method. The study protocol was approved by the Ethics Committee of Faculty of Medicine, Prince of Songkla University.

Samples preparation

Leftover serum specimens with various concentrations of creatinine and glucose were collected within 4 hours after obtaining the samples and stored at -20 ℃ until study. On the day of analysis, serum samples were thawed and some of them were mixed together if necessary to make the final glucose concentrations into 5 groups, namely <5.6, 5.6-11.1, 11.2-16.6, 16.7-22.2, and 22.3-27.8 mmol/L and each group consisted of 5 levels of creatinine concentration (<45, 45-88, 89-177, 178-265, and 266-354 µmol/L). We aimed to produce 5-10 samples in each cell depending on the creatinine and glucose concentrations of the collected specimens. Since the final concentrations of glucose and creatinine were not exactly the same as those expected from the calculation, the numbers in some cells were more or less than expected. All samples were analyzed in the same batch and on the same day.

Leftover urine specimens that were negative for glucose, protein, bilirubin, and blood were collected within 4 hours after obtaining the samples and stored at -20 ℃ until study. In order to get equal distribution, urine specimens were divided into 5 groups of creatinine concentration (<1,770, 1,770-3,540, 3,541-5,300, 5,301-7,960, and >7,960 µmol/L), each group consisted of at least 10 samples. On the day of analysis, urine samples were thawed and centrifuged at 3,000 rpm for 10 minutes. The supernatants were spiked with glucose powder to produce 5 aliquots with final glucose concentrations of 0, 167, 333, 500 and 666 mmol/L from each sample. All samples were analyzed in the same batch and on the same day.

Measurement method

Creatinine measurement by Jaffe kinetic method was performed by using an automated analyzer COBAS 8000 (Roche Diagnostics, Indianapolis, IN, USA). Urine samples were automatically diluted 1:20 with standard diluent before analysis. The intra-assay coefficient of variation at a mean concentration of 4,314 and 8,009 µmol/L was 2.66 and 2.87%, respectively.

Creatinine measurement by enzymatic method was performed by the same analyzer. Urine samples were automatically diluted 1:25 with standard diluent before analysis. The intra-assay coefficient of variation at a mean concentration of 4,270 and 8,000 µmol/L was 1.10 and 1.58%, respectively.

Glucose measurement by enzymatic method was performed by using an automated analyzer COBAS 8000 (Roche Diagnostics, Indianapolis, IN, USA). The samples were diluted 1:10 and 1:20 before analysis if the glucose levels were 167-333 mmol/L and 500-666 mmol/L, respectively. The intra-assay coefficient of variation at a mean concentration of 5.7 and 13.5 mmol/L was 0.98 and 1.39%, respectively.

Statistical analysis

Data were expressed as means ± SD, median (range) and percentages. Due to small numbers in each group, Kruskal Wallis test was used to analyze the differences of percent interference

among the groups followed by Bonferroni post hoc analysis. IBM SPSS Statistics for Windows, Version 28.0 (IBM corp., Armonk, NY, USA) was used for statistical analysis. The significance level was set at a p-value of 0.05.

RESULTS

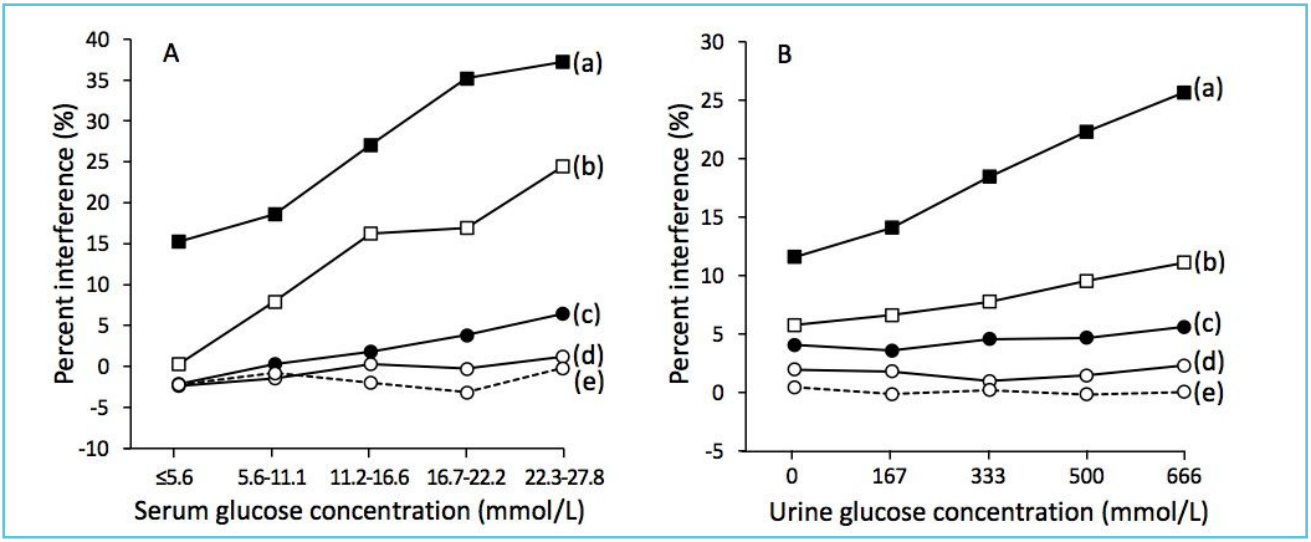
A total of 148 serum samples and 335 urine samples were analyzed. The glucose and creatinine concentrations of tested specimens in different groups are shown in Table 1. Table 2 and Figure 1A show the percent interference of glucose on serum creatinine measurement by the Jaffe kinetic method according to concentrations of creatinine and glucose. The percent interference was highest in the samples with creatinine of less than 45 µmol/L. The degree of interference was gradually decreased with increasing creatinine concentration. No glucose interference was found at serum creatinine of more than 177 µmol/L. At serum creatinine concentrations of less than 45 µmol/L, all glucose levels even less than 5.6 mmol/L interfered creatinine measurement by the Jaffe kinetic method. At creatinine concentrations of 45-88 µmol/L, glucose interference of creatinine measurement was found when glucose levels were 5.6 mmol/L or more. The effect of glucose interference with Jaffe creatinine measurement progressively increased with increasing glucose levels. As in serum specimens, the interference effects of glucose on Jaffe creatinine measurement in urine specimens showed a similar pattern (Table 3 and Figure 1B). At urine creatinine concentrations of less than 1,770 µmol/L, all glucose levels even no glucose interfered creatinine measurement by the Jaffe kinetic method. The degree of interference gradually decreased with increasing creatinine concentrations. At urine creatinine of 1,770-3,540 µmol/L, significant percent interference was found when glucose levels were 666 mmol/L. No glucose interference was found at urine creatinine of more than 3,540 µmol/L. The effect of glucose interference with Jaffe creatinine measurement progressively increased with increasing glucose levels. Although there was no glucose interference at creatinine concentration of 3,541-5,300

Table 1 Median (range) of glucose and creatinine concentrations of tested specimens in different groups

Group	Serum glucose mmol/L	Serum creatinine [a] µmol/L	Urine glucose mmol/L	Urine creatinine [a] µmol/L
1	4.7 (2.4-5.5)	37 (19-44)	0.2 (0-7.3)	1,266 (832-1,726)
2	7.9 (5.8-11.0)	57 (45-87)	178 (148-193)	2,545 (1,786-3,496)
3	13.9 (11.1-16.6)	135 (92-175)	350 (309-382)	4,517 (3,566-5,269)
4	18.9 (16.9-21.8)	220 (178-255)	527 (483-562)	6,752 (5,310-7,937)
5	24.7 (22.3-27.4)	304 (276-353)	701 (646-745)	10,096 (8,456-30,784)

[a] *Measurement by enzymatic method.*

Figure 1 Relationship between the mean percent interference of glucose on Jaffe creatinine measurement and glucose concentration



(A) in serum specimens, at 5 levels of creatinine: <45 µmol/L (a), 45-88 µmol/L (b), 89-177 µmol/L (c), 178-265 µmol/L (d), and 266-354 µmol/L (e); and

(B) in urine specimens, at 5 levels of creatinine: <1,770 µmol/L (a), 1,779-3,540 µmol/L (b), 3,541-5,300 µmol/L (c), 5,301-7,960 µmol/L (d), and >7,960 µmol/L (e).

Table 2 Mean ± standard deviation of percent interference of serum creatinine measurement by Jaffe kinetic method according to concentrations of creatinine and glucose

Serum creatinine (µmol/L)	Serum glucose (mmol/L)					*p* value (in row)
	<5.6	5.6-11.1	11.2-16.6	16.7-22.2	22.3-27.8	
<45	15.3±7.6[1,a]	18.6±8.8[1,2,a]	27.1±4.7[1,2,3,a]	35.2±11.5[2,3,a]	37.2±11.8[3,a]	0.01
45-88	0.3±1.7[1,b]	7.9±4.4[1,2,b]	16.2±4.3[2,3,b]	17.0±5.6[2,3,b]	24.4±13.5[3,a]	<0.001
89-177	-2.1±2.5[1,b]	0.3±1.8[1,2,b,c]	1.8±1.8[1,2,3,c]	3.8±5.4[2,3,c]	6.4±2.6[3,b]	0.001
178-265	-2.3±1.1[b]	-1.4±3.4[c]	0.3±2.2[c]	-0.3±1.4[c]	1.2±2.9[b]	0.212
266-354	-2.2±2.4[b]	-0.8±0.8[c]	-2.0±2.1[c]	-3.2±1.6[c]	-0.2±1.6[b]	0.112
p value (in column)	0.003	<0.001	<0.001	<0.001	<0.001	

Superscript numbers indicated intergroup Bonferroni post hoc comparisons in the same row. Values within rows not having a superscript number in common differ significantly (effective p < 0.05).
Superscript letters indicated intergroup Bonferroni post hoc comparisons in the same column. Values within columns not having a superscript letter in common differ significantly (effective p < 0.05).

Table 3 Mean ± standard deviation of percent interference of urine creatinine measurement by Jaffe kinetic method according to concentrations of creatinine and glucose

Urine creatinine (µmol/L)	Urine glucose (mmol/L)					*p* value (in row)
	0	167	333	500	666	
<1,770	11.6±4.3[1,a]	14.1±3.8[1,2,a]	18.4±5.8[2,3,a]	22.3±7.4[3,4,a]	25.6±8.5[4,a]	<0.001
1,770-3,540	5.8±3.6[1,b]	6.6±3.5[1,b]	7.7±4.2[1,2,b]	9.5±4.1[1,2,b]	11.1±4.7[2,b]	0.007
3,541-5,300	4.1±2.1[b,c]	3.6±2.1[b,c]	4.6±1.8[b,c]	4.7±2.3[c]	5.6±2.0[c]	0.192
5,301-7,960	2.0±1.9[c,d]	1.8±2.7[c,d]	1.0±1.4[c,d]	1.5±1.3[c,d]	2.3±1.8[c,d]	0.573
>7,960	0.5±1.7[d]	-0.1±13[d]	0.2±1.7[d]	-0.2±2.0[d]	0.1±1.5[d]	0.641
p value (in column)	<0.001	<0.001	<0.001	<0.001	<0.001	

Superscript numbers indicated intergroup Bonferroni post hoc comparisons in the same row. Values within rows not having a superscript number in common differ significantly (effective p < 0.05).
Superscript letters indicated intergroup Bonferroni post hoc comparisons in the same column. Values within columns not having a superscript letter in common differ significantly (effective p < 0.05).

µmol/L, the percent interference was small but significantly higher when compared with creatinine concentration of more than 7,960 µmol/L at all glucose levels.

DISCUSSION

The difference between serum and urine creatinine measurement by automated analyzers is due to the dilution process, which is required only for urine specimens. When creatinine concentration and specimen dilution were taken into account, this study found that no matter whether in serum or in urine specimens, the effects of glucose interference on Jaffe creatinine measurement were similar. In serum, glucose interference on Jaffe creatinine measurement was clearly found if creatinine concentrations were 88 µmol/L or less, which corresponds to less than 1,770 µmol/L in urine samples prior to 1:20 dilution. The effect of glucose interference was less in serum creatinine concentrations of 89-177 µmol/L, which corresponds to 1,770-3,540 µmol/L in urine specimens prior to 1:20 dilution.

The degree of interference was greater when glucose concentration was higher. The glucose interference effect was abolished when serum creatinine concentrations were more than 177 µmol/L, which corresponds to more than 3,540 µmol/L in urine samples prior 1:20 dilution. As with creatinine concentration, extremely high glucose concentrations in undiluted urine

specimens became similar to serum glucose concentrations after being 20 times diluted.

The glucose interference with Jaffe creatinine measurement in serum specimens were found consistently among studies [1-6]. The possible reason for this is that most of the serum creatinine concentrations in those studies were less than 177 µmol/L. Similar to previous studies [1,9], our study found that the higher the creatinine concentration, the less the glucose interference. An explanation is that glucose and creatinine react with alkaline picrate in Jaffe's reaction in a competitive manner. The high creatinine concentration would reduce the formation of glucose-picrate complexes which cause falsely high creatinine results. Interestingly, our study found that the interference with the Jaffe kinetic method at creatinine concentrations of less than 45 µmol/L was also found even when the glucose concentration was in normal range. Practically, serum creatinine in normal range determined by Jaffe kinetic method should therefore be interpreted with caution particularly in those with hyperglycemia as well as in those with low muscle mass, cirrhosis or malnutrition whose serum creatinine concentrations are usually low. However, Jaffe kinetic method is still a reliable tool for measuring creatinine at high concentrations without glucose interference.

There was only one study performed, by Watts and Pillay, that investigated the effect of glucose interference on Jaffe creatinine measurement in urine [10]. They studied the effects of glucose concentrations ranging from 5-320 mmol/L on 3 levels of creatinine concentrations (~2,000, ~10,000, and ~25,000 µmol/L) and found that glucose did not significantly influence the measurement of urine creatinine by Jaffe kinetic methods. Unfortunately, this study did not give the details of sample dilution during the process of creatinine measurement. If the urine samples were diluted 1:20 as in our study, their results were compatible with ours which showed that at urine creatinine concentration of 1,770-3,540 µmol/L, glucose concentrations of 500 mmol/L or less did not interfere with Jaffe creatinine measurement while the significant interferences were found at glucose concentration of 666 mmol/L. In addition, no interference effect of glucose was found at urine creatinine concentrations of more than 3,540 µmol/L.

Since serum creatinine concentrations in the general population are low, automated analyzers are designed to measure serum creatinine without dilution. In contrast, urine creatinine concentration is naturally much higher than serum creatinine concentration, therefore automated analyzers are programmed to dilute urine specimens 1:20 or 1:25 before analysis. Apart from creatinine, glucose and other substances in urine specimens that interfere with Jaffe creatinine measurement are also diluted. The dilution process can therefore explain the discrepancy of glucose interference with Jaffe creatinine measurement between in serum and in urine specimens which have similar glucose concentrations.

Although the dilution process can decrease or get rid of glucose interference with urine creatinine measurement by the Jaffe kinetic method, interpretation should be made with caution in patients receiving SGLT2 inhibitors. This class of drugs could increase urine glucose excretion up to 100 g/day [11]. After 1:20 dilution, these extremely high levels may remain high enough to interfere with Jaffe creatinine measurement, particularly in patients with urine creatinine concentration of 3,540 µmol/L or lower. Attenuation of albuminuria expressed as urine albumin to creatinine ratio in these patients may be overestimated.

This study had some limitations. As isotope-dilution mass spectrometry (IDMS) method was not available, the enzymatic method was used

as reference for studying the effect of glucose interference on Jaffe creatinine measurement. When compared with IDMS method, the enzymatic method has been found to show variation at a low creatinine concentration, which might affect the results of this study [12]. Extremely high glucose concentrations in urine samples were artificially created by adding glucose powder which might be different from urine samples collected from patients receiving SGLT2 inhibitors in terms of other interference substances. To confirm the effects of glucose interference on Jaffe creatinine measurement in urine, studies using samples collected from those receiving SGLT2 inhibitors are needed.

In conclusion, when creatinine concentration and specimen dilution were taken into account, the effects of glucose interference on Jaffe creatinine measurement were similar either in both serum and urine specimens, and was found when creatinine concentrations in serum or diluted urine were 177 µmol/L or less.

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Declaration of competing interests

All authors declare no conflicts of interest.

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Author contributions

PeC and SS designed the study, analyzed data, and wrote the manuscript. NB, PA, PhC, and YY were involved with sample collection and laboratory measurements. AB contributed to sample preparation. WS checked and approved the final data.

All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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