Effect of differences in serum creatinine estimation methodologies on estimated glomerular filtration rate

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INTRODUCTION

Serum creatinine is crucial in glomerular filtration rate (GFR) estimation. Various methods of measuring GFR have been developed, which vary in their ability to estimate the prevalence of chronic kidney disease (CKD) and predict consequences associated with CKD. The use of different laboratory devices also results in uncertainty in estimated GFR (eGFR). The purpose of our study was to discuss the effect of differences in laboratory devices on eGFR when performing serum creatinine measurements.

METHODS

163 participants aged 51.22 ± 18.66 years were enrolled during a community health screening programme conducted on 18 June 2011. Samples were sent to four different hospitals using four different devices to check serum creatinine by the Jaffe and enzymatic creatinine methods.

RESULTS

Using Roche Cobas Integra 400, Beckman LX20, Hitachi 7180 and Toshiba TBA – c8000, the proportion of the population with eGFR < 60 mL/min/1.73 m² was 11.04%, 6.75%, 20.25% and 20.86%, respectively. Moreover, 3.68% of the participants had eGFR < 60 mL/min/1.73 m² in the laboratory when Roche Cobas Integra 400 was used with the enzymatic creatinine method and compensated Jaffe method.

CONCLUSION

Although standardisation of serum creatinine measurement has been achieved by using isotope dilution mass spectrometry, differences in measurement devices still cause substantial bias in the overall results. This affects the application of GFR in the estimation of CKD progression and outcomes associated with CKD.

Keywords: glomerular filtration rate, laboratory devices, measurement, serum creatinine
in the estimation of GFR. However, the accuracy of GFR estimation is liable to be affected by variability such as differences in measurement devices, methodology and calculation as well as interference by chromogens or nonspecific proteins in serum. Hence, in this study, we compared results obtained using different devices and assays, and evaluated the effects of these differences on eGFR accuracy.

METHODS

A total of 163 participants aged 51.22 ± 18.66 years from Mudan Township, Pingtung County, Taiwan, were enrolled at a community health screening programme conducted on 18 June 2011. Their blood samples were collected between 0800 hours and 1100 hours. The participants were asked to fast for at least eight hours before blood sampling. Each participant’s blood was drawn using a vacutainer serum-separating tube and a blood collection tube, and the samples were stored at 4°C–8°C in a container before they were sent to a laboratory for examination. The samples were centrifuged at 3,000 revolutions per minute (rpm) for ten minutes. The serum was collected, divided and stored in 2-mL plastic containers. All specimens were stored at −20°C before further examination of Sn.

The samples were sent to four different laboratories with four different types of equipment to estimate Sn values using the Jaffe reaction and enzymatic creatinine methods. Samples were centrifuged at 3,000 rpm for five minutes. The serum was collected for analysis according to standard operation and inspection processes. To optimise Sn accuracy, samples were analysed for three days according to NKDEP creatinine standardisation guidelines using the Jaffe reaction and enzymatic creatinine methods. The chromogen used was calibrated through IDMS.

The study protocol was approved by the institutional review board of the Kaohsiung Medical University Hospital (KMUH-IRB-980457), Kaohsiung, Taiwan. Informed consent was obtained in written form and all clinical investigations were conducted according to the principles in the Declaration of Helsinki. The patients gave consent for the publication of clinical details.

The following devices that are often used in Taiwan were employed for this analysis – (a) Roche Cobas Integra 400 (Roche Diagnostics, Rotkreuz, Switzerland), reagent: Cobas Integra 400 Creatinine Plus Version 2 and Jaffe Gen.2 (Roche Creatinine plus/ Roche Diagnostics GmbH/Mannheim, Germany), standardised calibration reference materials: National Institute of Standards and Technology (NIST) Standard Reference Material (SRM) 914, proficiency testing: Bio-Rad External Quality Assurance Services (EQAS) assessment, where all EQAS programmes had American Association for Laboratory Accreditation ISO/IEC 17025:2010 Conformity Assessment – General Requirements for Proficiency Testing certification; (b) Beckman LX20 (Beckman Coulter, Brea, CA, USA), reagent: SYNCHRON® Systems (MUS3803000 Beckman Coulter Inc, Carlsbad, CA, USA), standardised calibration reference materials: CX/LX/LXi system analytical software, proficiency testing: College of American Pathologists assessment; (c) Hitachi 7180 (Hitachi, Tokyo, Japan), reagent: Wako (MJP1383000 Fujifilm Wako Pure Chemical Corporation Mie Plant, Mie-gun, Mie, Japan), standardised calibration reference materials: NIST SRM 914a, proficiency testing: Taiwan Accreditation Foundation (TAF) assessment; and (d) Toshiba TBA – c8000 (Toshiba, Tochigi, Japan), reagent: Denka Seiken (MJ0394000 Denka Seiken Co Ltd, Niigata, Japan), standardised calibration reference materials: NIST SRM 914a, proficiency testing: TAF assessment.

Analysis of variance was used for data analysis (Prism 5; GraphPad Software Inc, La Jolla, CA, USA). All values were expressed as mean ± standard deviation. The relationship between quantitative values were expressed using r², with an r² value nearer to 1 indicating stronger correlation. A value of p < 0.05 was considered significant. Sn level was expressed in mg/dL. eGFR was calculated using the following IDMS MDRD equation: 175 × (Sn−1.154 × (age−0.203 × (0.742 if female)) × (race factor)).

RESULTS

To ensure the precision of the analyser, pooled serum samples with known values of high creatinine level (Sn = 1.71 mg/dL) and low creatinine level (Sn = 0.55 mg/dL) were analysed in four different devices. During analysis of the variation (n = 5), the device was in a stable condition with the coefficient of variation being less than 5% (Table I). Table II lists the mean differences between Sn, eGFR and CKD staging. The results exhibited considerable variation. When eGFR < 60 mL/min/1.73 m², the values of eGFR obtained using different equipment varied from 2–5 times of the obtained value (Fig. 1). Moreover, 3.68% of the participants had eGFR < 60 mL/min/1.73 m² in the laboratory when Roche Cobas Integra 400 was used with the enzymatic creatinine method and compensated Jaffe method (Table I).

We selected the enzymatic creatinine method, which is standardised against IDMS as the gold standard, to investigate the difference in Sn measurement by the Jaffe reaction after standard calibration by IDMS, because of its higher specificity and freedom from interference by bilirubin and other medication. The results of Sn bias obtained using the Roche enzymatic, Roche compensated Jaffe, Roche Jaffe, Beckman Jaffe, Hitachi Jaffe

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Method</th>
<th>High/low coefficient of variation (%)</th>
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<tbody>
<tr>
<td></td>
<td>Intra-assay (n = 5)</td>
<td>Inter-assay (n = 5)</td>
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<tr>
<td>Roche Cobas Integra 400</td>
<td>Enzymatic Jaffe</td>
<td>0.93/0.57</td>
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<tr>
<td>Roche Cobas Integra 400</td>
<td>Compensated Jaffe</td>
<td>0.52/0.45</td>
</tr>
<tr>
<td>Roche Cobas Integra 400</td>
<td>Jaffe</td>
<td>0.93/0.57</td>
</tr>
<tr>
<td>Beckman LX20</td>
<td>Jaffe</td>
<td>0.92/0.82</td>
</tr>
<tr>
<td>Hitachi 7180</td>
<td>Jaffe</td>
<td>0.45/0.82</td>
</tr>
<tr>
<td>Toshiba TBA – c8000</td>
<td>Jaffe</td>
<td>0.91/1.19</td>
</tr>
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and Toshiba Jaffé methods were −0.008 ± 0.02, −0.165 ± 0.05, −0.026 ± 0.07, −0.18 ± 0.08 and −0.223 ± 0.06, respectively (Figs. 2–6). The mean bias values according to the percentage of eGFR were −1.06% ± 3.34%, 24.22% ± 9.67%, 2.91% ± 11.40%, 25.66% ± 11.60% and 31.04% ± 9.79%, respectively (Table II). In this study, the Roche enzymatic and Roche compensated Jaffé methods showed the strongest correlation ($r^2 = 0.9913$) with the smallest bias.

**DISCUSSION**

Although the $S_e$ values in each laboratory were calibrated using IDMS, considerable bias was observed when different equipment was used to measure $S_e$, affecting the accuracy of the eGFR values. This bias might be related to several factors. First, the calculation method, which traces back to the calibrated IDMS, is a major contributor to the bias. As creatinine calibration in the Jaffé and enzymatic methods merely traced back to the calibrated IDMS, the difference between these two groups should have been limited. However, in our analysis, considerable differences were observed in $S_e$ measurement (Figs. 3, 5 & 6). These differences were attributed to the intercept observed when standardised material was used for the standard calibration (e.g. NIST and SRM 914) and IDMS correlation. The intercept difference was not factored into the calculation. Therefore, we suggest performing compensation when using standardised material in tracing calibration IDMS.$^{13}$ Figs. 2 and 4 show that the bias between serum creatinine can be minimised after compensation.

Second, due to financial constraints and cost-saving policies, some laboratories adjust the parameters of their instruments and use lower-grade reagents and calibration reagents provided by different manufacturers. This variation adversely affects the accuracy of $S_e$ measurement. Previously, during a community screening programme in the Wanda district, Pingtung County, Taiwan, when the Toshiba TBA – c8000 was used to measure 172 $S_e$ samples using the Jaffé reaction, a total of 71 subjects were found with eGFR < 60 mL/min/1.73 m². However, when the Roche Cobas Integra 400 compensated Jaffé method was used, there were only seven subjects with eGFR < 60 mL/min/1.73 m². The variation in our result was attributed to differences in reagents and calculations performed by each device.

Third, the NKDEP suggests that the value of $S_e$ measurement should be reported to one decimal place when using the SI unit and to two decimal places when using the regular unit (mg/dL). As shown in Fig. 7, the difference of one decimal point caused considerable variation in the eGFR value.

The NKDEP suggests that after calibration of $S_e$ according to IDMS standardisation, the coefficient should be corrected from 186 to 175,$^{18}$ and that the uncertainty and analysis bias for $S_e$ must be < 8% and < 5%, respectively.$^{13}$ After calibration through IDMS, the difference between the Jaffé and enzymatic methods was minimised (Fig. 2). In Taiwan, approximately 75% of laboratories use open system equipment. If the $S_e$ reagent has not been compensated after calibration through IDMS, the results between equipments will vary greatly. Considerable variation among laboratory results will exist if the result cannot be

### Table II. Baseline characteristics of the participants (n = 163).

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Method</th>
<th>Age (yr)</th>
<th>Median</th>
<th>Maximum</th>
<th>Minimum</th>
<th>Cr (mg/dL)</th>
<th>eGFR (mL/min/1.73 m²)</th>
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<tr>
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<td>&lt; 15</td>
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<td>30–59</td>
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<td>60–89</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&gt; 90</td>
</tr>
<tr>
<td>Roche</td>
<td>Enzymatic</td>
<td>51.22 ± 18.66</td>
<td>1.85</td>
<td>1.98</td>
<td>0.39</td>
<td>0.75 ± 0.22</td>
<td>102.63 ± 31.98</td>
</tr>
<tr>
<td>Roche</td>
<td>Compensated Jaffé</td>
<td>1.86</td>
<td>1.99</td>
<td>0.4</td>
<td>0.74 ± 0.22</td>
<td>103.74 ± 31.19</td>
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</tr>
<tr>
<td>Roche</td>
<td>Jaffé</td>
<td>0.87</td>
<td>0.72</td>
<td>0.52</td>
<td>0.92 ± 0.22</td>
<td>79.65 ± 21.65</td>
<td></td>
</tr>
<tr>
<td>Beckman</td>
<td>Jaffé</td>
<td>0.90</td>
<td>0.90</td>
<td>0.31</td>
<td>0.78 ± 0.25</td>
<td>96.96 ± 25.52</td>
<td></td>
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<tr>
<td>Hitachi</td>
<td>Jaffé</td>
<td>0.90</td>
<td>0.90</td>
<td>0.5</td>
<td>0.93 ± 0.24</td>
<td>76.39 ± 16.70</td>
<td></td>
</tr>
<tr>
<td>Toshiba</td>
<td>Jaffé</td>
<td>0.6</td>
<td>0.6</td>
<td>0.6</td>
<td>0.97 ± 0.24</td>
<td>72.15 ± 13.70</td>
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Cr: creatinine; eGFR: estimated glomerular filtration rate; SD: standard deviation

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**Fig. 1** Graph shows the effect of measurement devices and methods on estimated glomerular filtration rate (eGFR) measurement. R-E: Roche (Cobas Integra 400) enzyme; R-J-C: Roche (Cobas Integra 400) compensated Jaffé; R-J: Roche Jaffé; B-J: Beckman (LX 20) Jaffé; H-J: Hitachi (7180) Jaffé; T-J: Toshiba TBA – c8000 Jaffé
Scientifically validated, and the accuracy of eGFR measurement will be adversely affected.

The Jaffe reaction can be influenced by endogenous chromogens such as acetone, fasting, lipidaemia, haemolysis and antibiotic drugs such as cephalosporins.\textsuperscript{(17,19)} In our study, \( S_{\text{cr}} \) calibration by the Jaffe reaction was affected by cephalaxin (Cefron\textsuperscript{®}). Currently, renal injuries caused by normal dosages of cephalaxin have not been documented. However, a greater creatinine reaction is observed when the Jaffe reaction is used, thereby leading to a higher \( S_{\text{cr}} \) measurement. Therefore, for patients
with cephalexin exposure, the enzymatic creatinine method should be used to measure S\textsubscript{cr} in order to avoid overestimation of S\textsubscript{cr} level. If the use of the enzymatic method is not feasible, blood S\textsubscript{cr} levels should be examined before prescription.

In conclusion, various large-scale epidemiological studies have been conducted in Taiwan for determining the incidence and prevalence of CKD. Thus, the accuracy and consistency of inter-laboratory S\textsubscript{cr} measurements are crucial. Although enzymatic creatinine methods should be used because they are unaffected by nonspecific proteins, haemolysis, vitamin C and drugs, most laboratories select the Jaffe reaction for S\textsubscript{cr} measurement because the enzymatic methods are expensive. The bias in S\textsubscript{cr} measurement can be minimised if compensation is performed, the conventional unit mg/dL is used, values are reported up to two decimal places, and the original parameters of the equipment are maintained. We suggest that the Taiwan Society of Nephrology develop a standardised S\textsubscript{cr} measurement to achieve more effective screening of CKD and conservation of medical resources. CKD is a silent disease that can develop substantially, and standardisation in S\textsubscript{cr} measurement and GFR estimation is important to facilitate accurate CKD detection.

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