Original Article

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Biochemistry Section

Effects of Jaffe and Enzymatic Creatinine Assays on eGFR Classification and Analytical Acute Kidney Injury Threshold Crossing: A Cross-sectional Method-comparison Study

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ABSTRACT

Introduction: Serum creatinine remains the routine marker for kidney-function assessment, but Jaffe and enzymatic assays can yield systematically different values. Such differences may alter estimated Glomerular Filtration Rate (eGFR) category assignment and may cross creatinine-based analytical thresholds used in Acute Kidney Injury (AKI) surveillance.

Aim: To compare Jaffe and enzymatic creatinine methods and determine their effect on serum creatinine, urine creatinine, creatinine clearance, Estimated Glomerular Filtration Rate (eGFR), Kidney Disease: Improving Global Outcomes (KDIGO), eGFR G-category (G1-G5) assignment, same-sample analytical Acute Kidney Injury (AKI)-threshold crossing, and exploratory calibration of Jaffe-derived eGFR.

Materials and Methods: This cross-sectional laboratory method-comparison study was conducted at the Department of Biochemistry in the clinical laboratory setting of NU Hospitals Pvt., Ltd., Bangalore, India, from January 2026 to March 2026. A total of 105 participants had paired serum Jaffe and enzymatic creatinine measurements; paired urine creatinine measurements were available for 98 participants. Correlation, regression, Bland-Altman agreement, eGFR G-category agreement, analytical AKI-threshold crossing, and exploratory calibration modelling were evaluated using Python and R.

Results: Mean age of the participants was 49.2±16.4 years, and 75 (71.4%) were male. Jaffe serum creatinine was higher than enzymatic serum creatinine by a mean±SD of 0.12±0.13 mg/dL, and Jaffe-derived eGFR was lower by a mean±SD of -9.0±11.0 mL/min per 1.73 m^2. Correlation was excellent for serum creatinine (Pearson's r=0.997), urine creatinine (r=0.998), creatinine clearance (r=0.977), and eGFR (r=0.973). eGFR G-category agreement was 70.5% (Cohen's kappa=0.63), with most discordance reflecting higher-risk categorisation by the Jaffe method. Nine participants (8.6%) crossed the same-sample analytical 0.3 mg/dL serum creatinine difference threshold. Exploratory calibration improved apparent eGFR category agreement to 74.3%, while 5-fold cross-validated agreement was 72.4%.

Conclusion: Jaffe and enzymatic creatinine measurements were highly correlated but not fully interchangeable. This analysis shows potentially clinically relevant shifts in eGFR category assignment and same-sample analytical AKI-threshold crossing when assay methods differ. Urine creatinine agreement was also strong but showed a positive Jaffe-minus-enzymatic bias. The creatinine assay method should therefore be documented during reporting, interpretation, method transition, and longitudinal follow-up.

Keywords: Chronic kidney disease, Creatinine, Estimated glomerular filtration rate, Kidney function tests

INTRODUCTION

Accurate assessment of kidney function is central to the diagnosis, staging, and monitoring of Chronic Kidney Disease (CKD) and AKI [1,2]. Serum creatinine is widely used because it is inexpensive, widely available, and incorporated into eGFR equations [3]. However, creatinine interpretation is affected by biological variation, calibration, and analytical method [2-4].

The Jaffe colorimetric method and enzymatic creatinine assays are the two most common laboratory approaches for creatinine estimation [1-3]. The Jaffe method is less specific and is susceptible to non creatinine chromogen interference [2,3]. Enzymatic assays generally provide greater analytical specificity and closer traceability to reference methods [2,4-6].

Even small creatinine differences can be clinically relevant because eGFR reporting uses categorical decision limits. Prior method-comparison studies have shown that moving between Jaffe and enzymatic methods can alter eGFR classification and kidney-disease detection, particularly near category boundaries [5-10]. Such differences may also influence interpretation when the 0.3 mg/dL creatinine threshold for AKI is approached [7].

To the best of our knowledge, limited laboratory-based evidence has evaluated paired serum and urine creatinine measurements by both Jaffe and enzymatic methods in the same participants while also quantifying KDIGO eGFR G-category reclassification and same-sample analytical AKI-threshold crossing [5-10]. These findings are important for laboratories that use, compare, or transition between assay platforms because clinicians may interpret numerical creatinine changes as biological deterioration when part of the difference is analytical. Therefore, the present study evaluated 105 participants with paired serum creatinine measurements and a urine-creatinine subset to quantify assay agreement, assess KDIGO eGFR G-category reclassification, identify analytical AKI-threshold crossing, and develop an exploratory calibration model aligning Jaffe-derived eGFR with enzymatic eGFR. The novelty of the revised analysis is the combined assessment of serum creatinine, urine creatinine,

creatinine clearance, eGFR category agreement, and same-sample analytical threshold crossing within one dataset.

MATERIALS AND METHODS

This was a cross-sectional laboratory method-comparison study conducted at the Department of Biochemistry, clinical laboratory setting of NU Hospitals Pvt., Ltd., Bangalore, Karnataka, India, from January 2026 to March 2026. Ethical approval was obtained from the Saveetha Medical College and Hospital Institutional Ethics Committee, Registration No. ECR-/724/Inst/TN/2015/RR-24 (IEC Reference Number: 003/01/2026/IEC/SMCH; approval date: 06 January 2026). Written informed consent was obtained from adult participants, and parent/guardian consent with participant assent was obtained where applicable for minors.

Inclusion criteria: Participants with available demographic, anthropometric, serum creatinine, and kidney-function calculation data were eligible. Participants were included in the primary serum analysis only when paired Jaffe and enzymatic serum creatinine values were available from the same sampling episode and processed within the same laboratory workflow. The urine-method comparison was performed in the subset with paired Jaffe and enzymatic urine creatinine values.

Exclusion criteria: Participants with missing paired serum creatinine data, haemolysed samples, insufficient serum or urine volume, incomplete urine collection, or active dialysis were excluded.

Sample size estimation: The sample size was estimated for detecting a minimum clinically relevant correlation of 0.30 between paired assay-derived kidney-function measures using Fisher z transformation:

$$n = \{(Z1\text{-}alpha/2 + Z1\text{-}beta)/\{0.5 \times \ln((1 + r)/(1 - r))\}\}.^2 + 3$$

With alpha=0.05, power=80%, and r=0.30, the minimum sample was approximately 85 participants. After allowing nearly 20% for exclusions or incomplete data, the required sample was 102; therefore, the final serum analytic sample of 105 participants was considered adequate for the primary method-comparison analysis [11].

Study Procedure

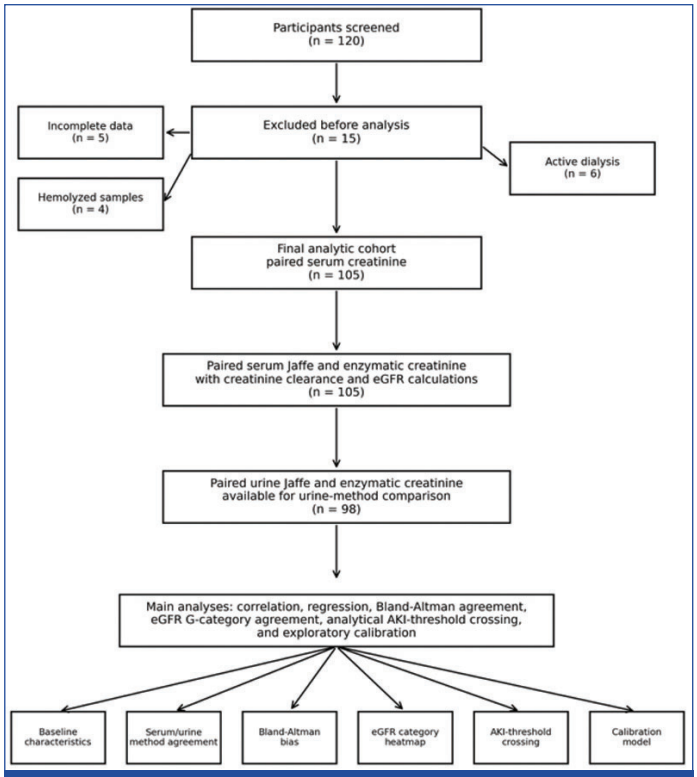
Consecutive eligible participants were screened. Demographic variables included age and gender; anthropometric variables included weight, height, and body mass index when available. Laboratory parameters included serum creatinine by Jaffe and enzymatic methods, urine creatinine by Jaffe and enzymatic methods where available, creatinine clearance, eGFR, eGFR G-category, same-sample analytical AKI-threshold crossing, and calibration-model outputs [Table/Fig-1].

Sample collection and handling: Blood and urine samples were collected during the same sampling episode according to local laboratory protocol. Serum creatinine was measured using Jaffe Colorimetric and enzymatic methods on calibrated automated analysers, and paired results were analysed from the same laboratory workflow. Urine creatinine was measured by both methods in the urine subset.

Derived variables and operational definitions: Creatinine clearance was calculated using the 24-hour urine clearance formula: creatinine clearance=(urine creatinine × urine volume) / (serum creatinine × collection time), with body-surface-area adjustment when applicable [12,13].

The eGFR was calculated using the 2021 CKD Epidemiology Collaboration equation without race [13]. eGFR G-categories were assigned according to kidney disease: Improving Global Outcomes (KDIGO) categories: G1 >=90, G2=60-89, G3a=45-59, G3b=30-44, G4=15-29, and G5 <15 mL/min per 1.73 m2 [12,13].

Analytical AKI-threshold crossing was operationally defined as a same-sample Jaffe-minus-enzymatic serum creatinine difference of ≥0.3 mg/dL, corresponding to the KDIGO serum creatinine change threshold used for AKI assessment when evaluated over time [14]. This definition was used only to evaluate assay-method impact on the KDIGO creatinine threshold and was not interpreted as clinical AKI because serial time-based clinical creatinine data and adjudicated AKI outcomes were not available.



[Table/Fig-1]: Study flow diagram showing screening, exclusions, final analytic cohort, paired serum analysis, paired urine-creatinine subset, and analysis sequence.

STATISTICAL ANALYSIS

Continuous variables were expressed as mean±standard deviation and median (interquartile range). Categorical variables were expressed as counts and percentages. Pearson's and Spearman correlation coefficients were calculated for paired Jaffe versus enzymatic serum creatinine, urine creatinine, creatinine clearance, and eGFR. Linear regression was used to estimate slope, intercept, and R2. Method agreement was assessed using Bland-Altman analysis with mean bias and 95% limits of agreement [15]. eGFR G-category agreement was summarised using a heatmap, percentage agreement, and Cohen's kappa [16]. Analytical AKI-threshold crossing was summarised overall and by sex and age group using Wilson 95% confidence intervals. Exploratory calibration modelling used multivariable ordinary least squares regression with enzymatic eGFR as the dependent variable and Jaffe eGFR, age, and sex as predictors. Model performance was summarised using R2, mean absolute error, root mean square error, apparent category agreement, and 5-fold cross-validated error. A two-sided p-value <0.05 was considered statistically significant.

RESULTS

A total of 105 participants were included in the paired serum-creatinine analysis, and paired urine creatinine measurements were available for 98 participants. Baseline demographic, anthropometric, biochemical, kidney-function, and eGFR category characteristics are summarised in [Table/Fig-2].

Variables	Mean±SD	Median (IQR)	n (%)
Total participants	–	–	105 (100)
Participants with paired serum creatinine	–	–	105 (100)
Participants with paired urine creatinine	–	–	98 (93.3)
Age (years)	49.2±16.4	49.0 (36.0–62.0)	–

<18 years	—	—	2 (1.9)
18–39 years	—	—	29 (27.6)
40–59 years	—	—	41 (39.0)
60–79 years	—	—	32 (30.5)
≥80 years	—	—	1 (1.0)
Male	—	—	75 (71.4)
Female	—	—	30 (28.6)
Weight (kg)	73.4±14.3	72.7 (62.7–83.3)	—
Height (cm)	162.9±9.4	164.0 (157.0–169.0)	—

[Table/Fig-2a]: Baseline demographic and anthropometric characteristics.
Data are presented as mean±SD, median (IQR), or n (%).

Variables	Mean±SD	Median (IQR)
Serum creatinine—Jaffe (mg/dL)	1.70±1.63	1.16 (0.91–1.77)
Serum creatinine—Enzymatic (mg/dL)	1.58±1.63	1.07 (0.78–1.65)
Δ Serum creatinine (Jaffe–Enzymatic)	0.12±0.13	0.09 (0.03–0.19)
Urine creatinine—Jaffe (mg/dL)	105.47±72.77	84.47 (48.83–155.05)
Urine creatinine—Enzymatic (mg/dL)	99.71±64.54	81.20 (48.93–145.72)
Δ Urine creatinine (Jaffe–Enzymatic)	5.76±9.47	3.53 (–1.37–10.28)

[Table/Fig-2b]: Comparison of Jaffe and Enzymatic creatinine measurements.
Δ denotes Jaffe minus enzymatic measurement.

Variables	Mean±SD	Median (IQR)	n (%)
Creatinine clearance—Jaffe (mL/min)	34.9±19.6	32.0 (21.0–45.0)	—
Creatinine clearance—Enzymatic (mL/min)	39.0±21.4	39.0 (25.0–50.0)	—
eGFR—Jaffe (mL/min/1.73 m²)	65.7±40.8	61.3 (38.2–82.5)	—
eGFR—Enzymatic (mL/min/1.73 m²)	74.6±45.4	66.3 (42.6–99.4)	—
KDIGO G1 (≥90)	—	—	34 (32.4)
KDIGO G2 (60–89)	—	—	26 (24.8)
KDIGO G3a (45–59)	—	—	17 (16.2)
KDIGO G3b (30–44)	—	—	9 (8.6)
KDIGO G4 (15–29)	—	—	15 (14.3)
KDIGO G5 (<15)	—	—	4 (3.8)

[Table/Fig-2c]: Kidney Function Parameters and KDIGO eGFR Categories.
eGFR = Estimated Glomerular Filtration Rate; KDIGO = Kidney Disease: Improving Global Outcomes

Mean age was 49.2±16.4 years, and 75 (71.4%) participants were male. Mean Jaffe-minus-enzymatic serum creatinine bias was +0.12±0.13 mg/dL, while mean Jaffe-minus-enzymatic eGFR difference was -9.0±11.0 mL/min per 1.73 m². Most participants were distributed across G1-G3a by enzymatic eGFR, although 19 participants were in G4-G5 categories.

Correlation and agreement between creatinine methods

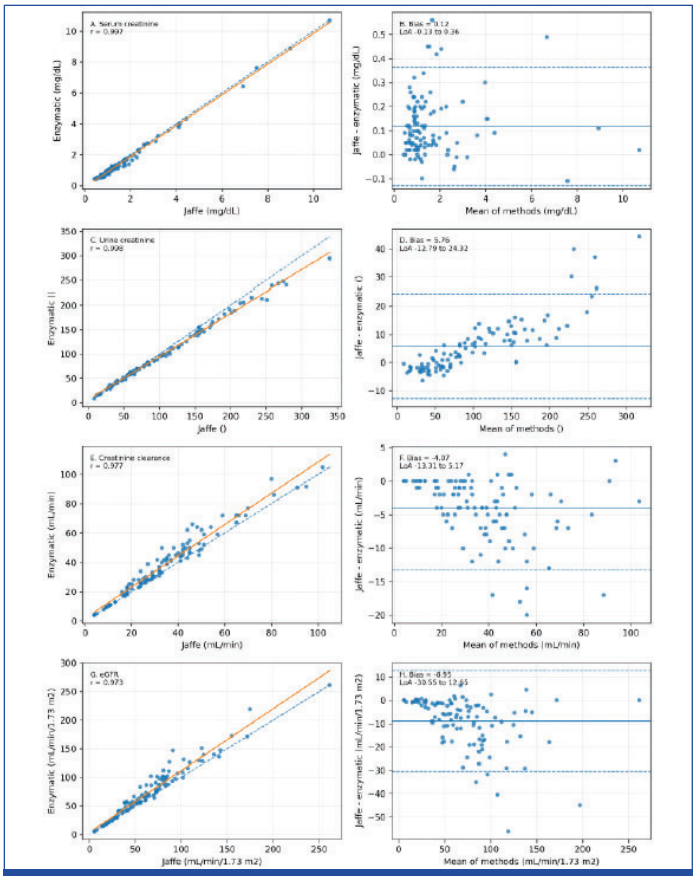
Correlation and regression analyses are summarised in [Table/Fig-3], while Bland–Altman agreement plots are presented in [Table/Fig-4].

All paired measures showed excellent correlation between Jaffe and enzymatic methods. Serum creatinine and urine creatinine had positive Jaffe-minus-enzymatic bias, whereas creatinine clearance and eGFR were lower when calculated using Jaffe creatinine. These findings support a strong linear association but incomplete interchangeability.

Bland-Altman analysis showed a mean serum creatinine bias of +0.12±0.13 mg/dL and a urine creatinine bias of +5.76±9.47 mg/dL. Creatinine clearance and eGFR demonstrated negative mean differences of -4.1±4.7 mL/min and -9.0±11.0 mL/min per 1.73 m2, respectively, reflecting lower function estimates when the Jaffe serum creatinine value was used [Table/Fig-5].

Parameters	n	Mean bias	SD of difference	Lower limit of agreement	Upper limit of agreement
Serum creatinine (mg/dL)	105	+0.12	0.13	-0.13	+0.36
Urine creatinine (mg/dL)	98	+5.76	9.47	-12.79	+24.32
Creatinine clearance (mL/min)	105	-4.1	4.7	-13.3	+5.2
eGFR (mL/min per 1.73 m2)	105	-9.0	11.0	-30.6	+12.6

[Table/Fig-3]: Correlation and regression between Jaffe and Enzymatic creatinine-related measures.
CI: Confidence interval; eGFR: estimated glomerular filtration rate; SD: Standard deviation. Mean delta represents Jaffe minus enzymatic value.



[Table/Fig-4]: Correlation and Bland-Altman plots comparing Jaffe and Enzymatic methods for serum creatinine, urine creatinine, creatinine clearance, and eGFR.

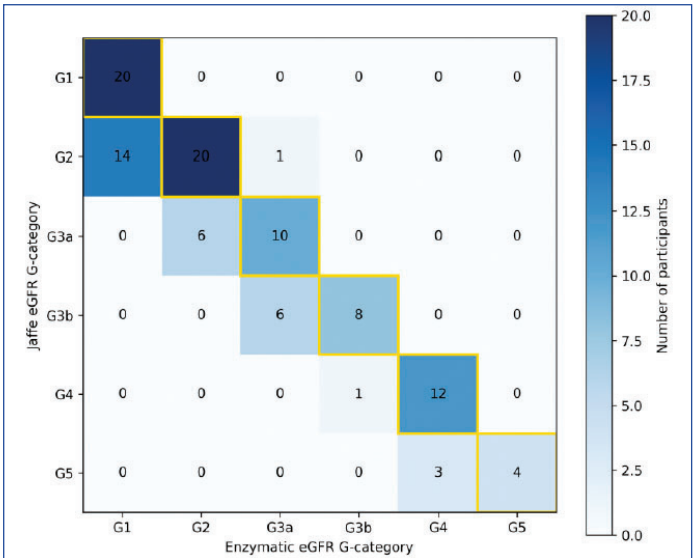
eGFR G-category agreement and reclassification

eGFR G-category classification was compared using Jaffe-derived and enzymatic-derived eGFR [Table/Fig-6].

Parameters	n	Mean bias	SD of difference	Lower limit of agreement	Upper limit of agreement
Serum creatinine (mg/dL)	105	+0.12	0.13	-0.13	+0.36
Urine creatinine					
(mg/dL)	98	+5.76	9.47	-12.79	+24.32
Creatinine clearance (mL/min)	105	-4.1	4.7	-13.3	+5.2
eGFR (mL/min per 1.73 m2)	105	-9.0	11.0	-30.6	+12.6

[Table/Fig-5]: Bland-Altman mean bias and 95% limits of agreement (Jaffe - Enzymatic).
Positive bias indicates higher Jaffe values. Limits of agreement were calculated as mean bias±1.96 standard deviations.

Rows represent Jaffe-derived eGFR G-category, columns represent enzymatic-derived eGFR G-category, and highlighted diagonal cells indicate agreement. Overall agreement was 70.5% and Cohen's kappa was 0.63.



[Table/Fig-6]: eGFR G-category reclassification heatmap.

Identical eGFR G-category classification occurred in 70.5% of participants. There were 31 discordant classifications, of which 30 were assigned to a higher-risk category by the Jaffe-derived eGFR and one to a lower-risk category. Discrepancies were mainly adjacent-category differences near decision thresholds.

Nine participants (8.6%) met the analytical AKI-threshold crossing definition. The frequency was higher among males and among participants aged 60-79 years. Because these were same-sample method differences, the finding reflects analytical impact on the KDIGO 0.3 mg/dL threshold rather than adjudicated clinical AKI [Table/Fig-7].

Categories	Threshold-crossing cases	n	% (95% CI)
Overall	9	105	8.6% (4.6-15.5)
Gender			
Male	8	75	10.7% (5.5-19.7)
Female	1	30	3.3% (0.6-16.7)
Age (in years)			
<18 years	0	2	0 (0.0-65.8)
18-39 years	0	29	0 (0.0-11.7)
40-59 years	3	41	7.3% (2.5-19.4)
60-79 years	6	32	18.8% (8.9-35.3)
≥80 years	0	1	0 (0.0-79.3)

[Table/Fig-7]: Frequency of analytical AKI-threshold crossing (delta serum creatinine >=0.3 mg/dL).
CI: Confidence interval. This table/Fig describes same-sample analytical threshold crossing only and should not be interpreted as clinical AKI.

The calibration model showed strong apparent fit (R2=0.952). Apparent eGFR G-category agreement increased from 70.5% to 74.3%, although 5-fold cross-validated agreement was 72.4%. This difference indicates that calibration may reduce method-related bias but should not be implemented clinically without external validation [Table/Fig-8].

Variables or metric	Estimate/value	95% CI or metric
Intercept	4.14	-5.25 to 13.53
eGFR-Jaffe coefficient	1.090	1.037 to 1.143
Age coefficient, per year	0.079	-0.053 to 0.211
Male coefficient	-6.99	-11.32 to -2.65
Model R2	0.952	-
Post-calibration mean absolute error	7.4 mL/min per 1.73 m2	-
Post-calibration root mean square error	9.9 mL/min per 1.73 m2	-
5-fold cross-validated MAE	8.2 mL/min per 1.73 m2	-
5-fold cross-validated RMSE	11.6 mL/min per 1.73 m2	-
CKD G-category agreement before calibration	70.5%	kappa=0.63
CKD G-category agreement after calibration	74.3%	kappa=0.67
5-fold cross-validated category agreement	72.4%	kappa=0.65
Bland-Altman bias before calibration	-9.0	LoA -30.6 to +12.6
Bland-Altman bias after calibration	+0.0	LoA -19.5 to +19.5

[Table/Fig-8]: Exploratory calibration model aligning Jaffe-derived eGFR with enzymatic eGFR.

DISCUSSION

The present study evaluated paired Jaffe and enzymatic creatinine measurements in 105 participants, with paired urine creatinine results available in 98. The principal finding was that the two creatinine methods were highly correlated but not fully interchangeable. Jaffe serum creatinine was higher than enzymatic serum creatinine, which translated into lower Jaffe-derived creatinine clearance and eGFR values. eGFR G-category agreement was substantial but incomplete, with 31 participants (29.5%) classified into different G-categories. Most discordance assigned participants to a higher-risk category by the Jaffe-derived eGFR, consistent with the positive Jaffe serum creatinine bias. Analytical AKI-threshold crossing occurred in 9 participants (8.6%). This outcome should be interpreted as same-sample assay-related threshold crossing, not clinical AKI, because serial creatinine change and clinical adjudication were not available.

The observed assay pattern is consistent with previous method-comparison studies showing that Jaffe assays can produce higher serum creatinine values than enzymatic assays because of non-creatinine chromogen interference. Syme NR et al., reported clinically meaningful effects after moving from Jaffe to enzymatic creatinine methodology, and Schmidt RL et al., demonstrated that assay choice can influence eGFR-based classification in outpatient settings [5,6].

The present eGFR category-shift pattern also aligns with evidence that method-related serum creatinine differences can affect kidney-disease detection and staging. Boss K et al., reported that differences between Jaffe and enzymatic creatinine measurements influenced kidney-disease and AKI classification, supporting the present finding that category shifts cluster near clinical decision thresholds [7].

The findings suggest that laboratories and clinicians should avoid assuming complete interchangeability between Jaffe and enzymatic creatinine methods, especially when patients are near eGFR category boundaries or when a change approaches the 0.3 mg/dL AKI threshold.

Reporting the analytical method and maintaining consistency of method during follow-up may reduce avoidable misclassification. Where method transition is unavoidable, local validation, calibration, and interpretive comments may help preserve continuity. The exploratory calibration model showed potential to reduce systematic bias, but its performance should be considered preliminary. Future multicentre studies should externally validate calibration equations across analysers, reagent lots, and diverse patient populations, and should include serial creatinine measurements with adjudicated clinical AKI outcomes before clinical implementation.

Limitation(s)

Several limitations require consideration. The sample size was adequate for the primary method-comparison objective but modest for subgroup analysis, particularly among participants aged ≥80 years. The urine-creatinine analysis included 98 paired observations rather than the full cohort. Clinical outcomes such as dialysis initiation, hospitalisation, mortality, and adjudicated AKI events were not available.

CONCLUSION(S)

Jaffe and enzymatic creatinine methods showed excellent correlation, but the results were not interchangeable. Jaffe creatinine was higher than enzymatic creatinine and produced lower creatinine clearance and eGFR estimates. These small assay-related differences influenced eGFR G-category assignment and crossed the same-sample analytical 0.3 mg/dL creatinine threshold in a minority of participants. Paired urine creatinine measurements also showed excellent correlation but positive Jaffe-minus-enzymatic bias.

Future studies should validate assay-calibration approaches across analysers, reagent lots, and patient populations and should incorporate serial clinical data to distinguish analytical threshold crossing from true AKI. Creatinine assay method should be documented and considered during eGFR interpretation, AKI-threshold interpretation, method transition, and longitudinal kidney-function follow-up.

Authors' contribution: All authors made substantial contributions to the work. Investigators based at NU Hospitals Pvt., Ltd., contributed to participant screening, sample collection, laboratory measurement, and data curation at the study site. Investigators based at Saveetha Medical College and Hospital contributed to study conception, protocol development, ethics coordination, statistical analysis, interpretation of findings, manuscript drafting, and critical intellectual revision. All authors reviewed and approved the final manuscript and agree to be accountable for the integrity and accuracy of all aspects of the work.

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- Manual Googling: Jun 20, 2026
- iThenticate Software: Jun 23, 2026 (1%)

ETYMOLOGY: Author Origin

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