PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

Title (Provisional)

Association between elevated serum REG Iα levels and eGFR decline in patients with chronic kidney disease: a cross-sectional study in eastern China

Authors

Huang, Nan; Zhu, Xiang Yun; Shu, Zhiyi; Chen, Sheng; Wu, Xiaodong; Wang, Hui; Huang, Xi; Hu, Xiuxiu; Sun, Jinfang; Chen, Pingsheng; Graf, Rolf; Bai, Jianling; Wang, Bin; Li, Ling

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VERSION 1 - REVIEW

Reviewer	**1**
Name	**Fujimaru, Takuya**
Affiliation	**Tokyo Medical and Dental University, Nephrology**
Date	**28-May-2024**
COI	**None.**

In this study, the authors analyzed the association between serum REG Iα levels and kidney function. Although this study shows the potential for a new biomarker of kidney dysfunction, several major and minor concerns are followings:

Major:

1. Multiple comparisons.

In Figure 1, the authors statistically compared and examined whether each biomarker value differed depending on the degree and severity of kidney dysfunction. Authors should explain what statistical analysis methods were used when comparing three or more groups. To avoid the problem of multiple comparisons, the authors should use Tukey's multiple comparison test, which tests for differences in population means for all group combinations. If the authors use the t-test to compare each of the two groups, Bonferroni correction is required for multiple comparisons.

2. Limitation of this study.

In this study, GFR values were not measured using gold standard methods such as urinary clearance of inulin or urinary clearance of 125I-iothalamate. The authors defined kidney

dysfunction by GFR estimated using serum Cr and investigated whether REG Iα was a better predictor of kidney dysfunction than serum Cr. The inability to assess true GFR appears to be a major limitation of this study.

3. Multicollinearity.

In Table 2, multiple logistic regression analysis was performed using different biomarkers of kidney dysfunction as covariates such as BUN, UA, myoglobin, Cys-C, and REG Iα. In general, when two or more of the predictors in a regression model are moderately or highly correlated, multicollinearity exists. Multicollinearity is a problem because it undermines the statistical significance of an independent variable. As shown in Figure 2, there was a correlation between REG Iα and each biomarker. Furthermore, it is easy to imagine that each biomarker is also correlated. Therefore, the authors should explain how they addressed the problem of multicollinearity.

Minor:

1. In the Results paragraph in the Abstract, "The regression analysis revealed" should be "The logistic regression analysis revealed.

2. For better understanding, the authors should clarify "kidney function" in the Results paragraph in the Abstract.

3. Cutoff value for serum REG Iα.

In the last paragraph in the Results section, how was the cutoff value for serum REG Iα determined? Was it a method using the Youden index?

I hope my comment will be helpful.

Reviewer	**2**
Name	**Sun, Likang**
Affiliation	**Tianjin University of Traditional Chinese Medicine**
Date	**15-Jul-2024**
COI	**No**

MS ID: bmjopen-2024-086874

To the manuscript, which entitled “Increased serum REG Iα is associated with eGFR decline in patients with chronic kidney disease”, Authors expect to understand the association between the increased serum level of REG Iα and the kidney function in patients with chronic kidney disease, thereby wish to establish serum REG Iα as a diagnostic biomarker for patients with chronic kidney disease. This is interesting topic, the design of this study is good, the inclusion criteria and the exclusion criteria were clear defined; the story is easy to follow, study followed logically, and all of the analysis are well presented. The statistical

analyses is appropriate; the references provided are appropriate and up to date; this study were permitted from the local Ethics committee, and registered. Even more, the limitation of this cross-sectional study were discussed. So that, this article shows promise for producing notable results and aligns with bmjopen's scope.

However, there are some augmentations needs to discuss/improve:

a) In discussion part, the article mentions that several studies indicate the renal tubules can secrete REG Iα and associate with kidney injury. KIM-1 is a widely accepted biomarker for renal tubular injury. Could the authors elaborate on the advantages of using serum REG Iα compared to serum KIM-1?

b) Are there variations in serum REG levels among patients with CKD of different etiologies? If so, it would be beneficial to conduct further subgroup analysis to explore its clinical implications.

c) The manuscript presents two regression analyses in the section "Relationship between serum REG Iα and kidney function". Are the study populations consistent across both regression analyses? There appears to be ambiguity in the manuscript regarding this. Please provide a detailed description of the research population in both the text (Ordinal Logistic Regression and Multivariate Logistic Regression) and table (Table 2). Clarify whether the populations represent all participants or only patients with chronic kidney disease.

Reviewer	**3**
Name	**Kelson, Zoe**
Affiliation	**University of Exeter, Mathematics**
Date	**16-Oct-2024**
COI	**None**

This cross-sectional study aims to demonstrate the relationship between levels of serum REG Iα and eGFR and to explore the efficiency of REG Iα in CKD detection.

Reviewer comments:

"The regression analysis revealed a significant association between serum REG Iα and eGFR (OR=1.737 [1.263-2.388], P = 0.001)." [Abstract]

Can the authors please clarify in the Abstract that a multivariate regression analysis adjusting for confounding has been applied (e.g. producing adjusted ORs)?

"The participants were enrolled from Zhongda Hospital between August 2022 and August 2023. "

Can the authors please comment on whether the included cohort can be considered to be representative for generalisability of the study findings?

"880 participants were enrolled in this study, with 220 non-CKD participants and 660 patients with CKD."

Can the authors please further the discussion on the included sample size?

"Demographics information was collected at baseline through questionnaires"

Can the authors please comment on the potential impact of self-reporting and recall bias in this study?

"Continuous data with normal distribution were summarized as mean ± standard deviation (SD), otherwise as median with interquartile range"

Can the authors please confirm how they assessed distributional assumptions (e.g. normality) for the choice of parametric or non-parametric statistical descriptors and methods?

"Correlation analyses were conducted to determine the association between REG Iα and kidney function."

and

"Spearman's rank correlation analyses and ordinal logistic regression was used to measure the associations between serum REG Iα and other biomarkers of kidney function. Multivariate logistic regression analysis was utilized to identify the independent factors of kidney dysfunction. "

Appropriate modelling methods have been applied by the authors.

Can the authors please specify the covariates included in models here?

"Receiver operating characteristic curves (ROC) were plotted to assess the ability of serum REG Iα in screening patients with CKD"

and

"The area under the receiver operating characteristic curve (AUC) was plotted to analyze the ability of serum Cys-C, KIM-1, and REG Iα to screen the patients with CKD, and detect the high and very-high risk patients."

The authors have suitably assessed model performance.

Can the other performance indicators examined please be specified in the methods section, such as sensitivity, specificity, NPV, PPV, and accuracy?

Can the authors please clarify in the methods how performace indicators were statistically compared (e.g. applying DeLong test to statistically analyse differences in AUC for instance)?

"Subgroup analysis in patients with CKD"

Can this subgroup analysis please be specified in the methods section?

Can it please be clarified how p-values were generated in Table 1, Figure 1, Figure 3, Supplementary data Table 3, Supplementary data Figure 2, and Supplementary data Figure 3 (i.e. specifying the stastical tests applied in each)?

"Although potential causal relationships can be identified through regression model analyses in this cross-sectional assessment, further prospective cohort follow-up is necessary to offer a more comprehensive understanding.

Our survey did not definitively identify the exact source of elevated REG Iα in patients with CKD. Therefore, further mechanistic studies should be conducted to investigate the origins of REG Iα in the situation of kidney impairment." [Strengths and limitations of this study]

and

"There are some limitations in this study. First, it is a cross-sectional assessment and further follow-up studies must be conducted to provide a more comprehensive understanding. Second, although robust biomarkers such as creatinine and Cys-C are already available for the diagnosis of CKD, the potential efficacy of REG Iα as a combined biomarker is anticipated to be revealed in subsequent large-scale analyses. Third, this study did not definitively identify the exact source of elevated REG Iα in patients with CKD. Therefore, further mechanistic studies should be conducted to investigate the origins of REG Iα in the situation of kidney impairment." [Discussion]

Can the study limitations please be expanded on in these sections? For instance, to further address residual confounding, causality, and generalisability?

Furthermore, given causality is not within scope of the study design, can the phrase 'potential causal relationships can be identified' please be removed?

Thanks for providing a copy of the STROBE checklist.

VERSION 1 - AUTHOR RESPONSE

Response to the comments of Reviewer # 1

We appreciate your insightful comments, which will help us enhance the design and robustness of our future studies.

Major:

Multiple comparisons.

Q 1. In Figure 1, the authors statistically compared and examined whether each biomarker value differed depending on the degree and severity of kidney dysfunction. Authors should explain what statistical analysis methods were used when comparing three or more groups. To avoid the problem of multiple comparisons, the authors should use Tukey's multiple comparison test, which tests for differences in population means for all group combinations. If the authors use the t-test to compare each of the two groups, Bonferroni correction is required

for multiple comparisons.

Answer.

We have conducted Tukey's multiple comparison test to examine the differences in biomarker values across the three or more groups, thereby avoiding the issue of multiple comparisons. The updated numerical results are as follows, and the corresponding figures have been revised in the manuscript accordingly. We appreciate the reviewers' insightful comments and believe these adjustments will enhance the clarity and rigor of our study.

Table1. Tukey's multiple comparison test for figure 1 in revised manuscript.

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold	Summary	Adjusted *P* Value
For Figure 1. A1. Serum Cys-C (mg/L)					
Non CKD vs. G1	0.02663	-0.4837 to 0.5369	No	ns	>0.9999
Non CKD vs. G2	-0.1934	-0.5716 to 0.1849	No	ns	0.6876
Non CKD vs. G3	-0.8461	-1.247 to -0.4450	Yes	****	<0.0001
Non CKD vs. G4	-1.702	-2.176 to -1.227	Yes	****	<0.0001
Non CKD vs. G5	-4.198	-4.601 to -3.795	Yes	****	<0.0001
G1 vs. G2	-0.2200	-0.6859 to 0.2459	No	ns	0.7555
G1 vs. G3	-0.8727	-1.357 to -0.3881	Yes	****	<0.0001
G1 vs. G4	-1.728	-2.275 to -1.181	Yes	****	<0.0001
G1 vs. G5	-4.225	-4.711 to -3.739	Yes	****	<0.0001
G2 vs. G3	-0.6527	-0.9956 to -0.3099	Yes	****	<0.0001
G2 vs. G4	-1.508	-1.935 to -1.082	Yes	****	<0.0001
G2 vs. G5	-4.005	-4.350 to -3.660	Yes	****	<0.0001
G3 vs. G4	-0.8556	-1.303 to -0.4085	Yes	****	<0.0001
G3 vs. G5	-3.352	-3.722 to -2.982	Yes	****	<0.0001
G4 vs. G5	-2.497	-2.946 to -2.048	Yes	****	<0.0001
For Figure 1. B1. Serum KIM-1 (pg/mL)					
Non-CKD vs. G1	-66.43	-145.5 to 12.65	No	ns	0.1570
Non-CKD vs. G2	-87.49	-152.6 to -22.34	Yes	**	0.0019
Non-CKD vs. G3	-111.2	-179.4 to -42.99	Yes	****	<0.0001
Non-CKD vs. G4	-99.80	-183.2 to -16.35	Yes	**	0.0088
Non-CKD vs. G5	-98.01	-171.9 to -24.11	Yes	**	0.0023
G1 vs. G2	-21.06	-88.23 to 46.11	No	ns	0.9471
G1 vs. G3	-44.78	-114.9 to 25.37	No	ns	0.4497
G1 vs. G4	-33.36	-118.4 to 51.67	No	ns	0.8720
G1 vs. G5	-31.58	-107.3 to 44.11	No	ns	0.8399
G2 vs. G3	-23.72	-77.67 to 30.23	No	ns	0.8077
G2 vs. G4	-12.30	-84.56 to 59.95	No	ns	0.9966
G2 vs. G5	-10.52	-71.50 to 50.46	No	ns	0.9964
G3 vs. G4	11.42	-63.61 to 86.45	No	ns	0.9980
G3 vs. G5	13.20	-51.05 to 77.45	No	ns	0.9918
G4 vs. G5	1.785	-78.45 to 82.02	No	ns	>0.9999
For Figure 1. C1. Serum REG Iα (ng/mL)					
Non-CKD vs. G1	-24.07	-86.10 to 37.96	No	ns	0.8780
Non-CKD vs. G2	-57.82	-108.5 to -7.147	Yes	*	0.0147
Non-CKD vs. G3	-96.19	-150.6 to -41.83	Yes	****	<0.0001
Non-CKD vs. G4	-195.3	-265.0 to -125.6	Yes	****	<0.0001
Non-CKD vs. G5	-495.5	-544.2 to -446.7	Yes	****	<0.0001
G1 vs. G2	-33.75	-98.55 to 31.05	No	ns	0.6724
G1 vs. G3	-72.12	-139.8 to -4.395	Yes	*	0.0292
G1 vs. G4	-171.2	-251.8 to -90.66	Yes	****	<0.0001
G1 vs. G5	-471.4	-534.7 to -408.1	Yes	****	<0.0001
G2 vs. G3	-38.37	-95.87 to 19.14	No	ns	0.3993
G2 vs. G4	-137.5	-209.7 to -65.29	Yes	****	<0.0001
G2 vs. G5	-437.6	-489.9 to -385.4	Yes	****	<0.0001
G3 vs. G4	-99.11	-173.9 to -24.29	Yes	**	0.0023

G3 vs. G5	-399.3	-455.1 to -343.4	Yes	****	<0.0001
G4 vs. G5	-300.2	-371.0 to -229.3	Yes	****	<0.0001
For Figure 1. D1. Serum Myoglobin (ng/mL)					
Non-CKD vs. G1	19.66	-0.5133 to 39.83	No	ns	0.0610
Non-CKD vs. G2	2.440	-16.58 to 21.46	No	ns	0.9991
Non-CKD vs. G3	-16.68	-33.33 to -0.02983	Yes	*	0.0493
Non-CKD vs. G4	-40.01	-59.68 to -20.34	Yes	****	<0.0001
Non-CKD vs. G5	-78.19	-92.72 to -63.67	Yes	****	<0.0001
G1 vs. G2	-17.22	-40.29 to 5.856	No	ns	0.2714
G1 vs. G3	-36.34	-57.50 to -15.18	Yes	****	<0.0001
G1 vs. G4	-59.67	-83.28 to -36.05	Yes	****	<0.0001
G1 vs. G5	-97.85	-117.4 to -78.32	Yes	****	<0.0001
G2 vs. G3	-19.12	-39.19 to 0.9485	No	ns	0.0721
G2 vs. G4	-42.45	-65.09 to -19.81	Yes	****	<0.0001
G2 vs. G5	-80.63	-98.98 to -62.29	Yes	****	<0.0001
G3 vs. G4	-23.33	-44.02 to -2.641	Yes	*	0.0167
G3 vs. G5	-61.51	-77.39 to -45.64	Yes	****	<0.0001
G4 vs. G5	-38.18	-57.20 to -19.17	Yes	****	<0.0001
For Figure 1. A2. Serum Cys-C (mg/L)					
Low risk vs. Moderate risk	-0.06694	-0.5558 to 0.4219	No	ns	0.9847
Low risk vs. High risk	-0.3447	-0.8560 to 0.1666	No	ns	0.3034
Low risk vs. Very-high risk	-1.672	-2.097 to -1.247	Yes	****	<0.0001
Moderate risk vs. High risk	-0.2778	-0.7697 to 0.2141	No	ns	0.4630
Moderate risk vs. Very-high risk	-1.605	-2.007 to -1.204	Yes	****	<0.0001
High risk vs. Very-high risk	-1.327	-1.756 to -0.8988	Yes	****	<0.0001
For Figure 1. B2. Serum KIM-1 (pg/mL)					
Low risk vs. Moderate risk	-18.53	-190.8 to 153.7	No	ns	0.9925
Low risk vs. High risk	-47.55	-227.8 to 132.7	No	ns	0.9038
Low risk vs. Very-high risk	-131.4	-280.9 to 18.11	No	ns	0.1072
Moderate risk vs. High risk	-29.02	-203.3 to 145.3	No	ns	0.9732
Moderate risk vs. Very-high risk	-112.9	-255.1 to 29.45	No	ns	0.1723
High risk vs. Very-high risk	-83.83	-235.7 to 68.05	No	ns	0.4834
For Figure 1. C2. Serum REG Iα (ng/mL)					
Low risk vs. Moderate risk	-13.11	-93.33 to 67.11	No	ns	0.9746
Low risk vs. High risk	-20.43	-104.4 to 63.52	No	ns	0.9225
Low risk vs. Very-high risk	-181.5	-251.5 to -111.5	Yes	****	<0.0001
Moderate risk vs. High risk	-7.319	-88.51 to 73.87	No	ns	0.9955
Moderate risk vs. Very-high risk	-168.4	-235.1 to -101.8	Yes	****	<0.0001
High risk vs. Very-high risk	-161.1	-232.2 to -90.01	Yes	****	<0.0001
For Figure 1. D2. Serum Myoglobin (ng/mL)					
Low risk vs. Moderate risk	-9.405	-53.84 to 35.03	No	ns	0.9472
Low risk vs. High risk	-3.524	-47.23 to 40.18	No	ns	0.9968
Low risk vs. Very-high risk	-57.41	-98.44 to -16.39	Yes	**	0.0020
Moderate risk vs. High risk	5.881	-22.50 to 34.27	No	ns	0.9502
Moderate risk vs. Very-high risk	-48.01	-72.07 to -23.95	Yes	****	<0.0001
High risk vs. Very-high risk	-53.89	-76.57 to -31.21	Yes	****	<0.0001
For Supplementary data Figure 2. A Serum REG Iα (ng/mL)					
Non-CKD vs. G1	-29.39	-95.72 to 36.94	No	ns	0.8033
Non-CKD vs. G2	-63.17	-120.4 to -5.966	Yes	*	0.0206
Non-CKD vs. G3	-96.86	-161.7 to -32.01	Yes	***	0.0003
Non-CKD vs. G4	-165.4	-247.5 to -83.36	Yes	****	<0.0001
Non-CKD vs. G5	-499.1	-555.8 to -442.3	Yes	****	<0.0001
G1 vs. G2	-33.78	-102.9 to 35.38	No	ns	0.7296
G1 vs. G3	-67.47	-143.1 to 8.143	No	ns	0.1114
G1 vs. G4	-136.1	-226.9 to -45.23	Yes	***	0.0003
G1 vs. G5	-469.7	-538.5 to -400.9	Yes	****	<0.0001
G2 vs. G3	-33.69	-101.4 to 34.06	No	ns	0.7142
G2 vs. G4	-102.3	-186.7 to -17.88	Yes	**	0.0075
G2 vs. G5	-435.9	-496.0 to -375.9	Yes	****	<0.0001

G3 vs. G4	-68.58	-158.3 to 21.18	No	ns	0.2467
G3 vs. G5	-402.2	-469.6 to -334.9	Yes	****	<0.0001
G4 vs. G5	-333.6	-417.7 to -249.6	Yes	****	<0.0001
For Supplementary data Figure 2. B Serum Myoglobin (ng/mL)					
Non-CKD vs. G1	12.63	-13.38 to 38.64	No	ns	0.7326
Non-CKD vs. G2	-6.662	-30.45 to 17.12	No	ns	0.9670
Non-CKD vs. G3	-18.06	-43.39 to 7.280	No	ns	0.3210
Non-CKD vs. G4	-37.21	-65.29 to -9.129	Yes	**	0.0024
Non-CKD vs. G5	-90.16	-112.3 to -68.02	Yes	****	<0.0001
G1 vs. G2	-19.29	-42.64 to 4.059	No	ns	0.1709
G1 vs. G3	-30.68	-55.61 to -5.757	Yes	**	0.0063
G1 vs. G4	-49.84	-77.55 to -22.12	Yes	****	<0.0001
G1 vs. G5	-102.8	-124.5 to -81.12	Yes	****	<0.0001
G2 vs. G3	-11.39	-33.99 to 11.20	No	ns	0.6999
G2 vs. G4	-30.55	-56.19 to -4.910	Yes	**	0.0092
G2 vs. G5	-83.50	-102.4 to -64.55	Yes	****	<0.0001
G3 vs. G4	-19.16	-46.24 to 7.930	No	ns	0.3297
G3 vs. G5	-72.10	-92.96 to -51.24	Yes	****	<0.0001
G4 vs. G5	-52.95	-77.07 to -28.83	Yes	****	<0.0001

Limitation of this study.

Q 2. In this study, GFR values were not measured using gold standard methods such as urinary clearance of inulin or urinary clearance of 125I-iothalamate. The authors defined kidney dysfunction by GFR estimated using serum Cr and investigated whether REG Iα was a better predictor of kidney dysfunction than serum Cr. The inability to assess true GFR appears to be a major limitation of this study.

Answer.

Thank you for your insightful comments regarding the limitations of our study. We wholeheartedly agree with your assessment that the inability to assess true Glomerular Filtration Rate (GFR) using gold standard methods, such as urinary clearance of inulin or 125I-iothalamate, is indeed a major limitation of our research.

We acknowledge that these gold standard methods provide the most accurate measurement of GFR. However, given the large sample size of our study, implementing these tests proved to be logistically challenging and resource-intensive. As a practical alternative, we chose to estimate GFR using serum creatinine (Cr), which is the most widely applied silver standard in clinical practice.

We understand that this approach may not capture the full picture of kidney function, but we believe it still offers valuable insights into the predictive value of REG Iα for kidney dysfunction. We are committed to addressing this limitation in future research and exploring more accessible methods that can approximate true GFR more closely.

We appreciate your understanding and constructive feedback, which will undoubtedly help us improve the quality of our work.

Multicollinearity.

Q 3. In Table 2, multiple logistic regression analysis was performed using different biomarkers of kidney dysfunction as covariates such as BUN, UA, myoglobin, Cys-C, and REG Iα. In

general, when two or more of the predictors in a regression model are moderately or highly correlated, multicollinearity exists. Multicollinearity is a problem because it undermines the statistical significance of an independent variable. As shown in Figure 2, there was a correlation between REG Iα and each biomarker. Furthermore, it is easy to imagine that each biomarker is also correlated. Therefore, the authors should explain how they addressed the problem of multicollinearity.

Answer.

Thank you for your insightful comments. To address this issue, we used a forward selection method during the logistic regression analysis. It allowed us to systematically and progressively select the most optimal subset of variables, thereby mitigating the potential impact of multicollinearity on our model's statistical significance.

Minor.

Q 4. In the Results paragraph in the Abstract, "The regression analysis revealed" should be "The logistic regression analysis revealed.

Answer.

Thanks for your detailed comment. We have revised the contents in the abstract and highlighted in red.

Q 5. For better understanding, the authors should clarify "kidney function" in the Results paragraph in the Abstract.

Answer.

Thank you for the suggestion. We have clarified the "kidney function" in the Abstract, and highlighted in red.

Q 6. Cutoff value for serum REG Iα. In the last paragraph in the Results section, how was the cutoff value for serum REG Iα determined? Was it a method using the Youden index?

Answer.

Yes, we used the best Youden index to determined the cutoff value for serum REG Iα.

Response to the comments of Reviewer # 2

Thank you for your valuable feedback and for highlighting important aspects of our study.

Q 1. In discussion part, the article mentions that several studies indicate the renal tubules can secrete REG Iα and associate with kidney injury. KIM-1 is a widely accepted biomarker for renal tubular injury. Could the authors elaborate on the advantages of using serum REG Iα compared to serum KIM-1?

Answer.

Thank you for your thoughtful comments and insightful questions. The advantages of serum REG Iα compared to serum KIM-1 can be summarized as follows.

1) Early Detection of Renal Injury. This paper demonstrated that REG Iα levels increase

significantly earlier than KIM-1, making it a superior marker for the early detection of renal injury. This early elevation allows for prompt intervention and potentially better clinical outcomes.

2) Sensitivity in Different Stages of CKD.

REG Iα was sensitive in distinguishing between different stages of CKD. Its ability to discriminate early from advanced stages of CKD provides valuable diagnostic and prognostic information.

3) Improved Diagnostic Performance. In the identification of patients with CKD , serum REG Iα has demonstrated better performance compared to serum KIM-1. Specifically, REG Iα exhibits higher AUC, sensitivity, and specificity, enhancing its diagnostic accuracy and clinical utility.

In summary, the advantages of serum REG Iα over serum KIM-1 primarily lie in its ability to detect renal injury earlier, its sensitivity in differentiating various stages of CKD, and its better diagnostic performance in identifying CKD patients. These features collectively underscore its potential as a potential biomarker for CKD.

Q 2. Are there variations in serum REG levels among patients with CKD of different etiologies? If so, it would be beneficial to conduct further subgroup analysis to explore its clinical implications

Answer.

Thank you for your valuable comment. We acknowledge the significance of investigating variations in serum REG Iα levels among patients with CKD of different etiologies and the potential clinical implications.

Due to the challenges associated with obtaining renal biopsy data, our team have endeavored to conduct a subgroup analysis within the available constraints. We examined serum REG Iα levels across 40 patients with membranous nephropathy, 40 patients with IgA nephropathy, and 80 patients with diabetic nephropathy. Regrettably, our analysis did not uncover any statistically significant differences in serum REG Iα levels among these subgroups. However, if the sample size is expanded with the severity of kidney disease is stratified and analyzed again, satisfactory results may be obtained. While we are disappointed that our current findings do not support the hypothesis of variations in serum REG levels based on CKD etiology, we believe that this information is still valuable for the scientific community.

Q 3. The manuscript presents two regression analyses in the section "Relationship between serum REG Iα and kidney function". Are the study populations consistent across both regression analyses? There appears to be ambiguity in the manuscript regarding this. Please provide a detailed description of the research population in both the text (Ordinal Logistic Regression and Multivariate Logistic Regression) and table (Table 2). Clarify whether the populations represent all participants or only patients with chronic kidney disease.

Answer.

Thank you for the valuable comment.
The study populations were not consistent across both regression analyses.
The ordinal multiple logistic regression was carried out in all participants.
The multivariate logistic regression analysis was carried out in patients with CKD.
We have added detailed instructions in the revised manuscript and highlighted in red.

Response to the comments of Reviewer # 3

We appreciate your continued interest in our work and your valuable input, which we believe will ultimately strengthen our research and its impact on clinical practice.

Q 1. "The regression analysis revealed a significant association between serum REG Iα and eGFR (OR=1.737 [1.263-2.388], P = 0.001)." [Abstract] Can the authors please clarify in the Abstract that a multivariate regression analysis adjusting for confounding has been applied (e.g. producing adjusted ORs)?

Answer.

Thank you for your thoughtful comments and suggestion.
The multivariate regression analysis in the study has indeed adjusted for confounding factors, including sex, diabetes, hypertension, and fasting blood glucose (FBG). We have revised the sentence in the Abstract to explicitly state these adjustments and highlighted in red.
"After adjusting for sex, diabetes, hypertension, and fasting blood glucose (FBG), the multivariate regression analysis revealed a significant association between serum REG Iα and eGFR (OR=1.737 [1.263-2.388], P = 0.001)."

Q 2. "The participants were enrolled from Zhongda Hospital between August 2022 and August 2023. "Can the authors please comment on whether the included cohort can be considered to be representative for generalisability of the study findings?

Answer.

Thank you for your insightful question.
The cohort was carefully selected from Zhongda Hospital, a tertiary institution with diverse patient demographics, ensuring a broad spectrum of CKD cases. While the study participants may not perfectly mirror the general population, the wide range of CKD severity and coexisting conditions enhances the generalizability of our findings. We believe the cohort provides robust insights applicable to a broader CKD population.

Q 3. "880 participants were enrolled in this study, with 220 non-CKD participants and 660 patients with CKD." Can the authors please further the discussion on the included sample size?

Answer.

Thank you for your insightful question regarding the sample size.
We conducted a sample size calculation to ensure our study had the necessary power to detect significant associations between serum REG Iα and eGFR. Using the following parameters. Expected proportion of participants with elevated serum REG Iα in CKD patients (estimated

from preliminary data). Desired power (80%) and significance level (0.05). Allowance for a two-sided test and finite population correction. The inclusion of 880 participants, comprising 220 non-CKD and 660 CKD patients, ensured a balanced representation across different CKD stages and comorbidities, enhancing the study's robustness and generalizability.

Based on preliminary data, we estimated the proportion of elevated serum REG Iα in the CKD group *P1*. The proportion of elevated serum REG Iα in the non-CKD group *P2*. Significance Level (α) set to 0.05 (two-tailed test). Power 1-β set to 80%. We used the following formula to calculate the sample size. Where *P1* is 0.3. Z (1-β) is the critical value for 80% power (approximately 0.84). n is the sample size for each group.

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2 \cdot (p_1(1-p_1) + p_2(1-p_2))}{(p_1 - p_2)^2}$$

Q 4."Demographics information was collected at baseline through questionnaires". Can the authors please comment on the potential impact of self-reporting and recall bias in this study?

Answer.

Thank you for the suggestion. We acknowledge the potential impact of self-reporting and recall bias in our questionnaire-based demographic data, especially considering the cognitive and emotional challenges common among CKD patients. Patients with CKD may be particularly vulnerable to self-reporting and recall biases due to several factors. Cognitive Impairment: CKD can be associated with cognitive dysfunction, including difficulties with memory and attention, which can affect the accuracy of self-reported information.

1) Symptom Complexity: Patients with CKD often experience multiple symptoms and comorbid conditions, making it challenging to accurately recall specifics over time.

2) Emotional and Psychological Factors: The stress and anxiety associated with CKD can influence how patients perceive and report their health status and experiences.

3) Health Literacy: Variations in health literacy can lead to misunderstandings of questions and subsequent misreporting of information.

4) Medication and Treatment Effects: The impact of medications and treatments on cognitive function and overall well-being can also introduce biases in reporting.

To mitigate these risks, we used standardized questionnaires and reinforced clear instructions. However, given the clinical complexity of CKD, our findings should be interpreted with caution. In future studies cross-verify self-reported data with objective clinical measures to enhance accuracy and reliability.

Q 5. "Continuous data with normal distribution were summarized as mean ± standard deviation (SD), otherwise as median with interquartile range"Can the authors please confirm how they assessed distributional assumptions (e.g. normality) for the choice of parametric or non-parametric statistical descriptors and methods?

Answer.

Thank you for your insightful question. We assessed the distributional assumptions for our

continuous variables using graphical methods, including Q-Q plots, as well as statistical tests such as the Shapiro-Wilk test. Variables were considered to have a normal distribution if the graphical evidence was consistent with normality and the Shapiro-Wilk test did not reject the null hypothesis of normality ($P > 0.05$). For variables that did not meet these criteria, we used non-parametric descriptors and methods.

Q 6. "Correlation analyses were conducted to determine the association between REG Iα and kidney function." and "Spearman's rank correlation analyses and ordinal logistic regression was used to measure the associations between serum REG Iα and other biomarkers of kidney function. Multivariate logistic regression analysis was utilized to identify the independent factors of kidney dysfunction. "Appropriate modelling methods have been applied by the authors. Can the authors please specify the covariates included in models here?

Answer.

Thank you for the considerable comment.

The study included age, BUN, UA, serum myoglobin, serum Cys-C, serum KIM-1/100, serum REG Iα/100 into ordinal multiple logistic regression model, while adjusting for sex, diabetes, hypertension, and FBG.

The multivariate logistic regression model also incorporates the above covariates.

Q 7. "Receiver operating characteristic curves (ROC) were plotted to assess the ability of serum REG Iα in screening patients with CKD" and "The area under the receiver operating characteristic curve (AUC) was plotted to analyze the ability of serum Cys-C, KIM-1, and REG Iα to screen the patients with CKD, and detect the high and very-high risk patients." The authors have suitably assessed model performance.

Can the other performance indicators examined please be specified in the methods section, such as sensitivity, specificity, NPV, PPV, and accuracy? Can the authors please clarify in the methods how performance indicators were statistically compared (e.g. applying DeLong test to statistically analyze differences in AUC for instance)?

Answer.

Thank you for the constructive suggestions.

We have now specified the other performance indicators examined, including sensitivity, specificity, NPV, and PPV in the methods section. Additionally, for the statistical comparison of performance indicators, we applied the DeLong test to analyze differences in AUC. The methods has been updated in revised manuscript and highlighted in red.

Q 8. "Subgroup analysis in patients with CKD" Can this subgroup analysis please be specified in the methods section? Can it please be clarified how p-values were generated in Table 1, Figure 1, Figure 3, Supplementary data Table 3, Supplementary data Figure 2, and Supplementary data Figure 3 (i.e. specifying the statistical tests applied in each)?

Answer.

Thank you for your valuable comments.

We have specified the "Subgroup analysis in patients with CKD" in methods section and highlighted in red.

Table 1. In baseline data analysis with two-group comparisons, we used appropriate statistical methods include t-tests for normally distributed continuous variables, Mann-Whitney U tests for non-normally distributed continuous variables, and chi-square or Fisher's exact tests for categorical variables. The choice of method depends on data type and distribution, ensuring accurate P-value calculations to assess group differences.

Figure 1 and Supplementary data Figure 2. We conducted Tukey's multiple comparison test to examine the differences in biomarker values across the three or more groups, thereby avoiding the issue of multiple comparisons.

Figure 3 and Supplementary data Table 3. We applied the DeLong test to statistically analyze AUC differences between receiver operating characteristic curves.

Supplementary data Figure 3. We used Mann-Whitney U tests for non-normally distributed continuous variables.

Q 9. "Although potential causal relationships can be identified through regression model analyses in this cross-sectional assessment, further prospective cohort follow-up is necessary to offer a more comprehensive understanding. Our survey did not definitively identify the exact source of elevated REG Iα in patients with CKD. Therefore, further mechanistic studies should be conducted to investigate the origins of REG Iα in the situation of kidney impairment."

[Strengths and limitations of this study] and "There are some limitations in this study. First, it is a cross-sectional assessment and further follow-up studies must be conducted to provide a more comprehensive understanding. Second, although robust biomarkers such as creatinine and Cys-C are already available for the diagnosis of CKD, the potential efficacy of REG Iα as a combined biomarker is anticipated to be revealed in subsequent large-scale analyses. Third, this study did not definitively identify the exact source of elevated REG Iα in patients with CKD. Therefore, further mechanistic studies should be conducted to investigate the origins of REG Iα in the situation of kidney impairment."

[Discussion] Can the study limitations please be expanded on in these sections? For instance, to further address residual confounding, causality, and generalisability?

Furthermore, given causality is not within scope of the study design, can the phrase 'potential causal relationships can be identified' please be removed?

Answer.

Thanks for your considerable comments.

First, we have deleted the phrase 'potential causal relationships can be identified' in the [Strengths and limitations of this study].

Second, the study limitations have expanded according to the constructive comments and highlighted in red.

VERSION 2 - REVIEW

Reviewer	**1**
Name	**Fujimaru, Takuya**
Affiliation	**Tokyo Medical and Dental University, Nephrology**
Date	**13-Dec-2024**
COI	

This second version of the paper is a great improvement, the authors are to be commended.

Reviewer	**3**
Name	**Kelson, Zoe**
Affiliation	**University of Exeter, Mathematics**
Date	**10-Dec-2024**
COI	

Thanks to the authors for responding to each comment in turn, providing clarification and undertaking revisions.

Can the following author responses please be further incorporated into the article?

"We conducted a sample size calculation to ensure our study had the necessary power to detect significant associations between serum REG Iα and eGFR. Using the following parameters. Expected proportion of participants with elevated serum REG Iα in CKD patients (estimated from preliminary data). Desired power (80%) and significance level (0.05). Allowance for a two-sided test and finite population correction. The inclusion of 880 participants, comprising 220 non-CKD and 660 CKD patients, ensured a balanced representation across different CKD stages and comorbidities, enhancing the study's robustness and generalizability. Based on preliminary data, we estimated the proportion of elevated serum REG Iα in the CKD group P1. The proportion of elevated serum REG Iα in the non-CKD group P2. Significance Level (α) set to 0.05 (two-tailed test). Power 1-β set to 80%. We used the following formula to calculate the sample size. Where P1 is 0.3. Z (1-β) is the critical value for 80% power (approximately 0.84). n is the sample size for each group."

and

"We acknowledge the potential impact of self-reporting and recall bias in our questionnaire-based demographic data, especially considering the cognitive and emotional challenges

common among CKD patients. Patients with CKD may be particularly vulnerable to self-reporting and recall biases due to several factors. Cognitive Impairment: CKD can be associated with cognitive dysfunction, including difficulties with memory and attention, which can affect the accuracy of self-reported information. 1) Symptom Complexity: Patients with CKD often experience multiple symptoms and comorbid conditions, making it challenging to accurately recall specifics over time. 2) Emotional and Psychological Factors: The stress and anxiety associated with CKD can influence how patients perceive and report their health status and experiences. 3) Health Literacy: Variations in health literacy can lead to misunderstandings of questions and subsequent misreporting of information. 4) Medication and Treatment Effects: The impact of medications and treatments on cognitive function and overall well-being can also introduce biases in reporting. To mitigate these risks, we used standardized questionnaires and reinforced clear instructions. However, given the clinical complexity of CKD, our findings should be interpreted with caution. In future studies cross-verify self-reported data with objective clinical measures to enhance accuracy and reliability."

and

"We assessed the distributional assumptions for our continuous variables using graphical methods, including Q-Q plots, as well as statistical tests such as the Shapiro-Wilk test. Variables were considered to have a normal distribution if the graphical evidence was consistent with normality and the Shapiro-Wilk test did not reject the null hypothesis of normality ($P > 0.05$). For variables that did not meet these criteria, we used non-parametric descriptors and methods"

and

"The study included age, BUN, UA, serum myoglobin, serum Cys-C, serum KIM-1/100, serum REG Iα/100 into ordinal multiple logistic regression model, while adjusting for sex, diabetes, hypertension, and FBG. The multivariate logistic regression model also incorporates the above covariates." [in to the 'Statistical analysis' section]

and

"Table 1. In baseline data analysis with two-group comparisons, we used appropriate statistical methods include t-tests for normally distributed continuous variables, MannWhitney U tests for non-normally distributed continuous variables, and chi-square or Fisher’s exact tests for categorical variables. The choice of method depends on data type and distribution, ensuring accurate P-value calculations to assess group differences. Figure 1 and Supplementary data Figure 2. We conducted Tukey’s multiple comparison test to examine the differences in biomarker values across the three or more groups, thereby avoiding the issue of multiple comparisons. Figure 3 and Supplementary data Table 3. We applied the DeLong test to statistically analyze AUC differences between receiver operating characteristic curves. Supplementary data Figure 3. We used Mann-Whitney U tests for non-normally

distributed continuous variables." [in the 'Statistical analysis' section, and in footnotes/captions for the Tables and Figures]

VERSION 2 - AUTHOR RESPONSE

Reviewer 2:

Q 1. In discussion part, the article mentions that several studies indicate the renal tubules can secrete REG Iα and associate with kidney injury. KIM-1 is a widely accepted biomarker for renal tubular injury. Could the authors elaborate on the advantages of using serum REG Iα compared to serum KIM-1?

Answer.

Thank you for your thoughtful comments and insightful questions. The advantages of serum REG Iα compared to serum KIM-1 can be summarized as follows.

1) Early Detection of Renal Injury. This paper demonstrated that REG Iα levels increase significantly earlier than KIM-1, making it a superior marker for the early detection of renal injury. This early elevation allows for prompt intervention and potentially better clinical outcomes.

2) Sensitivity in Different Stages of CKD.

REG Iα was sensitive in distinguishing between different stages of CKD. Its ability to discriminate early from advanced stages of CKD provides valuable diagnostic and prognostic information.

3) Improved Diagnostic Performance. In the identification of patients with CKD , serum REG Iα has demonstrated better performance compared to serum KIM-1. Specifically, REG Iα exhibits higher AUC, sensitivity, and specificity, enhancing its diagnostic accuracy and clinical utility.

In summary, the advantages of serum REG Iα over serum KIM-1 primarily lie in its ability to detect renal injury earlier, its sensitivity in differentiating various stages of CKD, and its better diagnostic performance in identifying CKD patients. These features collectively underscore its potential as a potential biomarker for CKD.

Answer:

The revised content has been incorporated into the discussion section (lines 367-378) of the revised manuscript.

Q 2. Are there variations in serum REG levels among patients with CKD of different etiologies? If so, it would be beneficial to conduct further subgroup analysis to explore its clinical implications.

Answer.

Thank you for your valuable comment. We acknowledge the significance of investigating variations in serum REG Iα levels among patients with CKD of different etiologies and the potential clinical implications.

Due to the challenges associated with obtaining renal biopsy data, our team have endeavored to conduct a subgroup analysis within the available constraints. We examined serum REG Iα levels across 40 patients with membranous nephropathy, 40 patients with IgA nephropathy, and 80 patients with diabetic nephropathy. Regrettably, our analysis did not uncover any statistically significant differences in serum REG Iα levels among these subgroups. However, if the sample size is expanded with the severity of kidney disease is stratified and analyzed again, satisfactory results may be obtained. While we are disappointed that our current findings do not support the hypothesis of variations in serum REG levels based on CKD etiology, we believe that this information is still valuable for the scientific community.

Answer:
The revised content has been integrated into the limitation part of the discussion section (lines 406-412) in the revised manuscript.

Reviewer 3:
Thanks to the authors for responding to each comment in turn, providing clarification and undertaking revisions. Can the following author responses please be further incorporated into the article?
"We conducted a sample size calculation to ensure our study had the necessary power to detect significant associations between serum REG Iα and eGFR. Using the following parameters. Expected proportion of participants with elevated serum REG Iα in CKD patients (estimated from preliminary data). Desired power (80%) and significance level (0.05). Allowance for a two-sided test and finite population correction. The inclusion of 880 participants, comprising 220 non-CKD and 660 CKD patients, ensured a balanced representation across different CKD stages and comorbidities, enhancing the study's robustness and generalizability. Based on preliminary data, we estimated the proportion of elevated serum REG Iα in the CKD group P1. The proportion of elevated serum REG Iα in the non-CKD group P2. Significance Level (α) set to 0.05 (two-tailed test). Power 1-β set to 80%. We used the following formula to calculate the sample size. Where P1 is 0.3. Z (1-β) is the critical value for 80% power (approximately 0.84). n is the sample size for each group."
Answer:
The revised content has been integrated into the study subjects of the methods section (lines 162-166) in the revised manuscript.

"We acknowledge the potential impact of self-reporting and recall bias in our questionnaire-based demographic data, especially considering the cognitive and emotional challenges common among CKD patients. Patients with CKD may be particularly vulnerable to self-reporting and recall biases due to several factors. Cognitive Impairment: CKD can be associated with cognitive dysfunction, including difficulties with memory and attention, which can affect the accuracy of self-reported information. 1) Symptom Complexity: Patients with CKD often experience multiple symptoms and comorbid conditions, making it challenging to accurately recall specifics over time. 2) Emotional and Psychological Factors: The stress and anxiety associated with CKD can influence how patients perceive and report their health status and experiences. 3) Health Literacy: Variations in health literacy can lead to misunderstandings of questions and subsequent misreporting of information. 4) Medication and Treatment Effects: The impact of medications and treatments on cognitive function and overall well-being can also introduce biases in reporting. To mitigate these risks, we used standardized questionnaires and reinforced clear instructions. However, given the clinical complexity of CKD, our findings should be interpreted with caution. In future studies cross-verify self-reported data with objective clinical measures to enhance accuracy and reliability."
Answer:
The revised content has been integrated into the limitation part of the discussion section (lines 420-427) in the revised manuscript.

"We assessed the distributional assumptions for our continuous variables using graphical methods, including Q-Q plots, as well as statistical tests such as the Shapiro-Wilk test. Variables were considered to have a normal distribution if the graphical evidence was consistent with normality and the Shapiro-Wilk test did not reject the null hypothesis of normality ($P > 0.05$). For variables that did not meet these criteria, we used non-parametric descriptors and methods"

Answer:
The revised content has been integrated into the statistical analysis of the methods section (lines 184-187) in the revised manuscript.

"The study included age, BUN, UA, serum myoglobin, serum Cys-C, serum KIM-1/100, serum REG Iα/100 into ordinal multiple logistic regression model, while adjusting for sex, diabetes, hypertension, and FBG. The multivariate logistic regression model also incorporates the above covariates." [in to the 'Statistical analysis' section]

Answer:
The revised content has been integrated into the statistical analysis of the methods section (lines 194-198) in the revised manuscript.

"Table 1. In baseline data analysis with two-group comparisons, we used appropriate statistical methods include t-tests for normally distributed continuous variables, MannWhitney U tests for non-normally distributed continuous variables, and chi-square or Fisher's exact tests for categorical variables. The choice of method depends on data type and distribution, ensuring accurate P-value calculations to assess group differences. Figure 1 and Supplementary data Figure 2. We conducted Tukey's multiple comparison test to examine the differences in biomarker values across the three or more groups, thereby avoiding the issue of multiple comparisons. Figure 3 and Supplementary data Table 3. We applied the DeLong test to statistically analyze AUC differences between receiver operating characteristic curves. Supplementary data Figure 3. We used Mann-Whitney U tests for non-normally distributed continuous variables." [in the 'Statistical analysis' section, and in footnotes/captions for the Tables and Figures]

Answer:
The revised content has been integrated into the statistical analysis of the methods section (lines 187-191, 202-204) in the revised manuscript. And have revised the footnotes/captions for the Tables and Figures.
