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Complete List of Authors:	Huang, Nan; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Key Laboratory of Environmental Medicine Engineering of Ministry of Education Zhu, Xiang; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute Shu, Zhiyi; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute Chen, Sheng; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute Wu, Xiaodong; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute Wang, Hui; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute Huang, Xi; Southeast University Zhongda Hospital, Department of Endocrinology, Zhongda Hospital, School of Medicine Hu, Xiuxiu; Southeast University, Department of Pathology, School of Medicine Sun, Jinfang; Southeast University, Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health Chen, Pingsheng; Southeast University, Department of Pathology, School of Medicine Graf, Rolf; University Hospital Zurich, Department of Surgery Bai, Jianling; Nanjing Medical University, Department of Biostatistics, School of Public Heath Wang, Bin; Southeast University Zhongda Hospital, Department of Nephrology Li, Ling; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Key Laboratory of Environmental Medicine Engineering of Ministry of Education
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Increased serum REG Iα is associated with eGFR decline in patients with chronic kidney disease

Author

Nan Huang[1, 2, 3#], Xiangyun Zhu[1, 3#], Zhiyi Shu[1, 3#], Sheng Chen[1, 3], Xiaodong Wu[1, 3], Hui Wang[1, 3], Xi Huang[1], Xiuxiu Hu[4], Jinfang Sun[5], Pingsheng Chen[4], Rolf Graf[6], Jianling Bai[7*], Bin Wang[8*], Ling Li[1, 2, 3*]

Address

1. Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China.

2. Key Laboratory of Environmental Medicine Engineering of Ministry of Education, Southeast University, Nanjing, Jiangsu, 210009, China.

3.Pancreatic Research Institute, Southeast University, Nanjing, Jiangsu 210009, China.

4. Department of Pathology, School of Medicine, Southeast University, Nanjing 210009, China.

5. Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu 210009, China.

6. Department of Visceral and Transplantation Surgery, University Hospital of Zurich, Zurich, 8091, Switzerland.

7. Department of Biostatistics, School of Public Heath, Nanjing Medical University, Nanjing, Jiangsu, 211166, China.

8. Department of Nephrology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China.

Correspondence

# These authors have contributed equally to this article.

* Address correspondence to:

1. Ling Li*: M.D.

Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China. (e-mail: lingli@seu.edu.cn);

2. Bin Wang*: Ph.D.

Department of Nephrology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China. (email: wangbinhewei@126.com);

3. Jianling Bai*: Ph.D.

Department of Biostatistics, School of Public Heath, Nanjing Medical University, Nanjing, Jiangsu, 211166, China. (email: baijianling@njmu.edu.cn);

Abstract

Objective

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

Design

A cross-sectional study.

Setting

Conducted in Zhongda Hospital between August 2022 and August 2023.

Participants

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

Methods

Correlation analyses were conducted to determine the association between REG Iα and kidney function. Receiver operating characteristic curves (ROC) were plotted to assess the ability of serum REG Iα in screening patients with CKD.

Results

In CKD group, the levels of serum REG Iα (125.54 [60.28-303.39] ng/mL) were significantly higher compared to those in non-CKD group (24.62 [14.09-37.32] ng/mL, $P < 0.001$). Serum REG Iα exhibited a positive relationship with serum creatinine (Scr), cystatin C (Cys-C), and kidney injury molecular-1(KIM-1), while showed a negative relationship with eGFR. The regression analysis revealed a significant association between serum REG Iα and eGFR (OR=1.737 [1.263-2.388], $P = 0.001$). Furthermore, levels of serum REG Iα were found to gradually increase with the decline of kidney function ($P < 0.001$). Serum REG Iα was recognized to play a role in screening CKD patients, with an AUC of 0.860 (0.813-0.899), providing a sensitivity of 71.63%, a specificity of 86.89%, a PPV of 94.30%, and an NPV of 46.85%. Additionally, serum REG Iα had an AUC of 0.769 (0.712-0.819) in screening patients at high and very-high risk for CKD according to KDIGO risk stratification, exhibiting significantly higher sensitivity compared to serum Cys-C and KIM-1 (82.80% vs 75.16% and 36.94%).

Conclusions

This study provided evidence that levels of serum REG Iα were notably elevated in patients with CKD and exhibited a strong association with kidney function. The REG Iα might serve as an important biomarker for CKD.

Trial registration

The study was approved by the ethics committee of Zhongda Hospital (2022ZDSYLL204-P01) and was in compliance with the Helsinki Declaration. The study had a clinical study registration number of ChiCTR2300072247.

Data availability statement

The data underlying this article is available from the corresponding author under reasonable request.

Patient and public involvement statement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

Strengths and limitations of this study

- We utilized different logistic regression analyses to adjust for various confounding factors and investigate the relationship between serum REG Iα and kidney function.
- This study was the first time to apply KDIGO risk stratification for subgrouping in the CKD population, analyzing the potential predictive ability of serum REG Iα in renal function decline and the risk of CKD progression.
- 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.

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Competing interests statement

The authors declare that they have no competing interests.

Keywords

Regenerating protein Iα, Chronic kidney disease, Biomarker, Kidney function, Risk stratification;

Abbreviations

List of abbreviations	
Regenerating protein Iα	REG Iα
Diabetic kidney disease	DKD
Chronic kidney disease	CKD
Receiver operating characteristic curves	ROC
Cystatin C	Cys-C
Kidney injury molecular-1	KIM-1
Area under the ROC	AUC
End stage kidney disease	ESKD
Serum creatinine	Scr
Blood urea nitrogen	BUN
Estimate glomerular filtration rate	eGFR
Urine albuminuria creatine ratio	UACR
Kidney disease improving global outcomes	KDIGO
Pancreatic stone protein	PSP
Chronic kidney disease epidemiology collaboration	CKD-EPI
Unilateral ureteral obstruction	UUO
Uric acid	UA
Fast blood glucose	FBG
Cardiovascular disease	CVD
Standard deviation	SD

Introduction

Chronic kidney disease (CKD) has a broad range of underlying etiologies and variable progression rates, [1,2] and may become the fifth leading cause of death worldwide by 2040.[3] The endpoint of CKD is end stage kidney disease (ESKD) in which 90% of kidney function reduced, making it impossible to sustain life over the long term.[2] The high prevalence, low detection rate, severe outcomes, and substantial medical costs of CKD make it a significant global health concern.[4] Early prevention, detection, and treatment can lead to better outcomes and prevent ESKD progression.

Biomarkers such as serum creatinine (Scr), blood urea nitrogen (BUN), estimate glomerular filtration rate (eGFR), and urine albuminuria creatine ratio (UACR) are routinely used to assess the severity of CKD.[5-8] In 2012, Kidney Disease: Improving Global Outcomes (KDIGO) has taken the eGFR and UACR to create risk stratification strategies, assisting in the tailored management of CKD and guiding treatment for those at a higher risk of progression.[9] New biomarkers like, cystatin C (Cys-C), kidney injury molecular-1 (KIM-1), and β2-microglobuline have shown great application potential in enhancing the precision of early-stage CKD screening, either independently or in conjunction.[2,3,10,11] However, most biomarkers failed to match clinical expectations.[12-15] Few biomarkers can both detect and assess risk stratification effectively in patients with CKD. Thus, discovering a biomarker can screen kidney function decline and evaluate CKD progression risk is crucial.

Regenerating protein Iα (REG Iα) is a 16 kDa protein primarily secreted by the pancreas and intestine,[16] also known as pancreatic stone protein (PSP).[17] The involvement of REG Iα plays a role in the processes of cellular proliferation and regeneration.[18,19] Recent researchers have identified the presence of REG Iα in patients with various kidney diseases.[20,21] Our previous studies also have indicated that serum levels of REG Iα elevate in patients with diabetic kidney disease (DKD), which are concordant with the finding of H. Sobajima.[22-24] These evidences indicate that REG Iα may serve as a biomarker of kidney insufficiency.

In this study, we analyzed the association between serum REG Iα levels and kidney

function, and further assessed its potential as a screening tool for CKD and as a biomarker of kidney function.

Methods

Study subjects

The participants were enrolled from Zhongda Hospital between August 2022 and August 2023. The study was approved by the ethics committee (2022ZDSYLL204-P01), with a clinical study registration of ChiCTR2300072247. Informed consent was acquired from all participants.

The inclusion criteria were as follows: (1) non-CKD participant: age older than 18 years; (2) CKD patients: age older than 18 years and diagnosed with CKD in accordance with the guidelines in 2012.[1] The exclusion criteria were as follows: (1) enrolled in another trial; (2) acute kidney injury; (3) patients with ESKD who are undergoing renal replacement therapy; (4) pregnancy; (5) active infection; (6) acute or chronic inflammation of the gastrointestinal system and pancreas; (7) history of gastrointestinal or pancreatic resections; (8) cancer; (9) mental disorders.

eGFR was calculated in accordance with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[25] CKD groups was classified using the method proposed by the U.S. National Kidney Foundation. The risk stratification of CKD progression was defined in accordance with KDIGO risk stratification guideline.[26,27]

Data collection and quality assessment

Demographics information was collected at baseline through questionnaires. All participants have undergone 12-h fasting and taken about 3mL of peripheral blood sample. The blood samples were centrifuged directly for 15 min at a rotating speed of 3,500 rpm, and the upper serum was sucked up within 6-h. The serum was immediately frozen in refrigerator (-80℃). Clinical biochemical parameters were extracted from the clinical laboratory of Hospital, such as Scr, BUN, uric acid (UA), Cys-C, Myoglobin, UACR, and fast blood glucose (FBG). The laboratory implements internal and external quality control procedures directed by a Chinese Quality Control Laboratory. The serum REG Iα levels were determined by a double antibody sandwich ELISA, as

previously described.[28] Serum KIM-1 was detected by an ELISA kit (KE00136) from Proteintech.

Statistical analysis

Statistical analyses were conducted using SPSS 20.0, Med-Calc, and GraphPad Prism 8.0. Continuous data with normal distribution were summarized as mean ± standard deviation (SD), otherwise as median with interquartile range. For categorical variables, the frequency with a percentage of each category was calculated. 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. 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. A *P* value of <0.050 using two-tailed tests was considered statistically significant.

Results

Baseline characteristics of the study population

Overall, 880 participants were finally enrolled, with 220 non-CKD participants and 660 patients with CKD (Supplementary data Figure 1). Differences in age, complication diseases (diabetes and hypertension), kidney function biomarkers (Scr, BUN, UA, eGFR, Cys-C, and KIM-1), and serum myoglobin were observed between patients with CKD and non-CKD participants (all $P < 0.001$, Table 1). Serum REG Iα levels were notably higher in patients with CKD (125.54 [60.28-303.39] ng/mL), than in non-CKD participants (24.62[14.09-37.32] ng/mL, $P < 0.001$, Table 1). Serum Cys-C, REG Iα, and myoglobin showed increasing trends as eGFR levels decreased (Figure 1: A1, C1, and D1). There were significant differences in serum REG Iα among each group ($P < 0.001$, Figure 1: C1). A significant difference in serum KIM-1 was found between the non-CKD group and G1 group ($P < 0.010$, Figure 1: B1). Two reanalyzes balanced the effects of CVD on the distribution of serum REG Iα and myoglobin (supplementary data Figure 2), and the effects of diabetes on patients with CKD (Supplementary data

Figure 3).

Relationship between serum REG Iα and kidney function

This study explored the relationship between serum REG Iα levels and kidney function biomarkers. In figure 2, Spearman correlation analyses demonstrated that serum REG Iα was positively correlated with Scr (r = 0.753, $P < 0.001$), BUN (r = 0.733, $P < 0.001$), serum Cys-C (r = 0.678, $P < 0.001$), and serum KIM-1 (r = 0.217, $P < 0.001$), and negatively correlated with eGFR (r = -0.789, $P < 0.001$). The comprehensive correlations between serum REG Iα and various markers were presented in supplementary data Table 1. In ordinal logistic regression analysis, eGFR values were used as a grade-dependent variable in the model. The results showed that serum REG Iα/100 levels was markedly associated with eGFR (OR=1.737 95% CI: 1.263-2.388, $P = 0.001$, Table 2).

Subgroup analysis in patients with CKD

To investigate the relationship between serum REG Iα and the risk of CKD progression, 256 CKD patients were included in the sub-research and were classified by eGFR and UACR levels in accordance with KDIGO risk stratification guideline.[1] The patients were divided into four groups to assess the progression and prognostic, namely, low risk (18.00%), moderate risk (20.70%), high risk (17.20%) and very-high risk (44.10%, supplementary data Table 2). We found that levels of serum Cys-C elevate gradually from 1.22 (1.01, 1.43) mg/L in the moderate risk group to 2.57 (1.94, 3.53) mg/L in the very-high risk group ($P < 0.010$), however, no significant difference was found between the low risk group and moderate risk group ($P > 0.050$, Figure 1: A2). The levels of serum REG Iα elevate gradually from 48.86 (34.18-78.28) ng/mL in the low risk group to 184.38 (108.81-314.71) ng/mL in the very-high risk group ($P < 0.010$). Significant differences were observed in serum REG Iα levels among the groups ($P < 0.010$), except for the moderate risk group and high risk group ($P > 0.050$, Figure 1: C2). The very-high risk group had the highest serum myoglobin level among the four groups ($P < 0.010$), however, there were no significant differences among other three groups ($P > 0.050$, Figure 1: D2).

The multiple logistic regression analysis demonstrated that serum REG Iα/100 was an independent influencing factor for patients with CKD who are at high and very-high risk (OR=1.799, 95% CI: 1.088-2.975, $P = 0.022$, Table 2).

Ability of serum REG Iα in screening patients with kidney dysfunction

In evaluating the potential application of serum REG Iα as a screening tool for patients with CKD and its ability to differentiate the risk stratification, we performed receiver operating characteristic curve (ROC) analyses (Figure 3 and supplementary data Table 3). The AUC for serum REG Iα to detect patients with CKD was 0.860 (95% CI: 0.813-0.899) as well as serum creatinine (0.850, 95% CI: 0.801-0.890) and Cys-C (0.842, 95% CI: 0.793-0.883), with a cutoff value of 70.82 ng/mL, sensitivity of 71.63%, specificity of 86.89%, positive predictive value (PPV) of 94.30%, and negative predictive value (NPV) of 46.85%. By contrast, serum KIM-1 had a lower AUC than serum REG Iα, measuring 0.714 (95% CI: 0.656-0.767, $P < 0.001$). Serum myoglobin had the lowest AUC among the five biomarkers, measuring 0.642 (95% CI: 0.581-0.699, $P < 0.001$). Furthermore, we analyzed the ability of serum Cys-C, KIM-1, and REG Iα in distinguishing high and very-high risk patients with CKD in accordance with the KDIGO risk stratification. As shown in supplementary data Table 3, serum Cys-C had an AUC of 0.865 (95% CI: 0.817-0.904, $P < 0.010$), which was the highest AUC among the three markers. Serum REG Iα had an AUC of 0.769 (95% CI: 0.712-0.819), showing better performance than serum KIM-1 ($P < 0.010$). The cutoff value of serum REG Iα was 76.05 ng/mL, and the sensitivity, specificity, PPV, and NPV were 82.80%, 62.63%, 77.38%, and 69.32%, respectively. Notably, serum REG Iα had a significantly higher sensitivity than serum Cys-C and KIM-1.

Discussion

This study was the first time to investigate the whole CKD population, and confirm the upregulation of serum REG Iα in patients with CKD. Initially, the serum levels of REG Iα showed a gradually increasing trend with the decrease of eGFR. Second, serum REG Iα was negatively correlated with eGFR and positively correlated with Scr, BUN, serum Cys-C, and serum KIM-1 levels. Third, serum REG Iα was an independent risk factor

for high and very-high risk patients in accordance with the KDIGO risk stratification. Finally, serum REG Iα played an important role in screening patients with CKD and detecting high and very-high risk patients in CKD.

REG Iα was a low molecular weight (16 kDa) protein initially discovered in pancreas, where it exhibited a remarkable ability to inhibit the formation of calcium carbonate stones in pancreatic ducts.[29] Hence, REG Iα was also called pancreatic stone protein (PSP) at the time.[30] Immunocytochemical analyses indicated that REG Iα protein was overexpressed in impaired kidney, with predominant localization observed in proximal tubules and thick ascending limbs of Henle's loops.[20] Then, some studies declared that urine PSP (REG Iα) and *REG Iα* gene might indicate kidney tubular dysfunction and potentially serve as an early biomarker of DKD detection and progression.[22,31,32] Our previous studies provided compelling evidence, that serum REG Iα levels were notably elevated in patients with type 2 diabetes mellitus who had kidney dysfunction. And we also observed that pregnant women with early renal dysfunction exhibited similar elevations in serum REG Iα levels.[24,33] Notably, the serum REG Iα levels remained significantly elevated when the scope of this study was expanded to the entire CKD population.

We incorporated myoglobin (17.6 kDa)[34] to investigate whether the elevated level of serum REG Iα represents a universal phenomenon of accumulation in CKD. The myoglobin was identified as a biomarker for acute myocardial ischemia and rhabdomyolysis.[35] Some studies indicated that serum myoglobin levels increase in kidney impairment. This accumulation was primarily attributed to slower clearance in CKD.[36] We showed that serum levels of myoglobin have no differences between non-CKD participants and patients with early stage of CKD (group G1and group G2). And there was a gradual increase in patients with medium to end-stages of CKD (group G3 to group G5). The possible reason was as follows: in the early stages of kidney injury, compensatory glomerular hypertrophy with hyperfiltration state was known to exist. This mechanism allowed some low molecular weight protein to be filtered through the glomerular basement membrane, resulting in no discernible difference during the early

stage of CKD (eGFR ≥ 60 mL/min per 1.73 m^2), and even appearing as a transient decrease. With progression to medium to end-stages of CKD (eGFR < 60 mL/min per 1.73 m^2), the glomerular basement membrane thickened and led to significant decline in glomerular filtration function, ultimately causing proteins accumulation in serum. [37] The serum levels of REG Iα showed a gradual increase in medium to end-stages of CKD, which was similar to the rise observed in serum myoglobin levels. However, it exhibited a significant increase in the early stage of CKD, which was different with serum myoglobin. This suggested that, in addition to the sensitive accumulation of serum REG Iα as a low molecular weight protein, there might be a specific production when it comes to kidney impairment.

The etiology underlying the upregulation of REG Iα production in patients with CKD remains elusive. Two potential mechanisms are considered as follows. First, REG Iα resists apoptosis and promotes cell proliferation in different inflammation situations.[38-42] Studies have shown that cytokines such as IL-6 can increase the proliferation of REG Iα, which is involved in cell regeneration and repair.[42,43] In CKD, the occurrence and progression of the disease are closely related to the expression of multiple inflammatory cytokines, including IL-1, IL-6, and TNF-α. [37,44,45] Under the stimulation of chronic inflammation, different types of renal cells secrete REG Iα locally to participate in kidney anti-apoptosis and proliferation and against kidney fibrosis in the development of CKD. The secreted REG Iα enters the circulation through reabsorption by kidney tubules, resulting in a significant increase in serum REG Iα levels. Thus, REG Iα might serve as an inflammatory factor involved in kidney diseases. Second, REG Iα is primarily synthesized in the pancreas and released into the circulation.[18,46] A hypothesis suggests that a cross-talk mechanism exists between the pancreas and kidney when renal damage occurs. This interaction may prompt the pancreas to produce more REG Iα in response to kidney injury, resulting the elevation in serum. Although the exact cause of REG Iα upregulation remains unclear, these two proposed mechanisms provided potential insights into the relationship between REG Iα and CKD.

At present, the assessment of CKD generally focused on glomerular filtration capacity,

which is characterized by Scr, UACR, and eGFR.[2] The stabilities of these factors are compromised by age, dietary intake, and physical activity. In general, the performance of eGFR is poor for populations outside of North America, Australia, and Europe, and accuracy decreases at the extremes in the distribution of age, and body composition.[7] Therefore, many new biomarkers of kidney impairment have been discovered. Cys-C is produced by all nucleated cells at a steady rate and freely filtered by the kidney.[47] The accumulation of its levels is observed in case of glomerular filtration dysfunction, with limited impact and strong stability.[48] Eventually, serum Cys-C is incorporated into the eGFR equation as a reliable and sensitive indicator of kidney function. The sensitivity of serum Cys-C is heightened in the early stages of CKD compared to creatinine.[49,50] Another biomarker is KIM-1,[51,52] which is secreted following kidney proximal tubular injury, and has emerged as a sensitive and specific biomarker of acute kidney injury and CKD progression.[53-56] In this study, serum REG Iα strongly correlated with serum creatinine and Cys-C, and had a similar performance to serum creatinine and Cys-C in detecting patients with CKD. The serum levels of REG Iα were more likely related to glomerular filtration capacity according to the correlation analyses, although the mechanism of its production and degradation or excretion in CKD remained unclear. We indicated that serum REG Iα might be more sensitive than serum creatinine and Cys-C in detecting ultra-early stage of kidney dysfunction. Similar phenomenon was observed in distribution analyses in KDIGO risk stratification. The KDIGO risk classification was widely used in evaluating the progression and prognosis risk in patients with CKD.[57-60] Serum levels of REG Iα gradually elevated with higher KDIGO risk stratification categories. And it emerged as an independent risk factor for patients with CKD categorized as high and very-high risk. Our findings provided a new insight that serum REG Iα performed better than serum KIM-1 in screening patients with CKD and detecting high and very-high risk patients with CKD. Moreover, serum REG Iα displayed a highest sensitivity in identifying high and very-high risk patients with CKD. These results highlight the potential application of serum REG Iα as a valuable biomarker in the screening of patients with CKD and the assessment of CKD

risk.

We observed that patients with CKD were older than non-CKD participants, and they had higher rates of diabetes and hypertension. This finding was consistent with the typical etiology of CKD. Globally, diabetes and hypertension are recognized as the primary causes of CKD.[2] Diabetes accounts for 30-50% of all CKD cases and affects approximately 285 million adults worldwide. A consistent finding in observational studies shows that the increased risk of developing is associated with blood pressure control. These findings were consistent with the expected proportion of CKD etiology in our study.

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.

Conclusion

This study provided compelling evidence that serum REG Iα was significantly upregulated in the patients with CKD and strongly associated with kidney function. Serum REG Iα emerged as a significant biomarker for detecting kidney function decline.

Authors' contributions

Ling Li conceptualized the study; Rolf Graf provided the excellent technical support for this study; Bin Wang reviewed and edited the manuscript; Nan Huang and Xiangyun Zhu were responsible for the data analysis and wrote the original draft. Nan Huang, Sheng Chen, Xiaodong Wu, Hui Wang, Zhiyi Shu and Xi Huang were responsible for inclusion of population, collection of samples and data, and the experiments. Jianling Bai and Jinfang Sun were responsible for guidance on data analysis. Pingsheng Chen and Xiuxiu Hu were responsible for analysis of kidney biopsy tissues from CKD patients.

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Tables

Table 1. Clinical characteristics of study population at baseline examination.

	Non-CKD Participants	CKD Patients	*P* Value
Number	220	660	-
DEMOGRAPHICS			
Age (years)	53 (40-62)	62 (50-72)	< 0.001
Sex (male, %)	111 (50.45)	366 (55.45)	0.212
Smoking (yes, %)	41 (18.64)	142 (21.52)	0.141
Drinking (yes, %)	30 (13.64)	84 (12.73)	0.823
COMPLICATION DISEASES			
Diabetes (yes, %)	75 (34.09)	350 (53.03)	< 0.001
Hypertension (yes, %)	65 (29.55)	501 (75.91)	< 0.001
Cardiovascular disease (CVD, yes, %)	44 (20.00)	149 (22.58)	0.453
LABORATORY MEASUREMENTS			
Fast blood glucose (FBG, mmol/L)	5.45 (4.99-6.67)	5.34 (4.57-7.11)	0.008
Serum creatinine (Scr, μmol/L)	64.00 (55.25-76.00)	126.00 (83.00-418.50)	< 0.001
Blood urea nitrogen (BUN, mmol/L)	5.20 (4.30-6.28)	10.30 (7.00-18.60)	< 0.001
Serum uric acid (UA, μmol/L)	301.00 (256.00-365.25)	354.00 (290.00-443.00)	< 0.001
Estimated glomerular filtration rate (eGFR, mL/min per 1.73 m^2)	102.37 (95.73-112.36)	44.03 (10.96-77.09)	< 0.001
Serum cystatin C (Cys-C, mg/L)	1.10 (0.99-1.24)	1.79 (1.23-3.50)	< 0.001
Serum kidney injury molecular-1 (KIM-1, pg/mL)	186.28 (57.22-266.88)	247.72 (175.10-334.13)	< 0.001
Serum Myoglobin (ng/mL)	46.81 (33.49-58.00)	64.04 (35.96-112.51)	< 0.001
Serum regenerating protein Iα (REG Iα, ng/mL)	24.62 (14.09-37.32)	125.54 (60.28-303.39)	< 0.001

CKD: chronic kidney disease; The data were presented in quartiles.

554 Table 2. The logistic regression analyses showing the relationship between variables
555 and kidney function.

	Ordinal Logistic Regression#		Multivariate Logistic Regression*	
	***P* Value**	**OR (95% CI)**	***P* Value**	**OR (95% CI)**
Age[a]	0.050	1.020 (1.001-1.041)	0.009	0.966 (0.942-0.992)
BUN[b]	< 0.001	1.266 (1.165-1.376)	< 0.001	1.440 (1.216-1.706)
UA[c]	0.337	1.001 (0.999-1.004)	0.085	1.003 (1.000-1.007)
Serum Myoglobin[d]	0.165	1.005 (0.998-1.013)	N/A	N/A
Serum Cys-C[e]	< 0.001	6.784 (4.016-11.460)	0.071	1.853 (0.949-3.620)
Serum KIM-1/100[f]	0.133	1.069 (0.980-1.167)	0.122	1.243 (0.943-1.639)
Serum REG Iα/100[d]	0.001	1.737 (1.263-2.388)	0.022	1.799 (1.088-2.975)

556 #: The ordinal multiple logistic regression showing variables independently associated with eGFR levels
557 in all participants.
558 *: The multivariate logistic regression analysis identified the independent influencing factors for high
559 and very-high risk patients with CKD in accordance with KDIGO risk stratification.
560 The two logistic regression analyses have been adjusted the sex, diabetes, hypertension, and FBG.
561 FBG: fast blood glucose, BUN: blood urea nitrogen, UA: serum uric acid, Cys-C: cystatin C, KIM-1:
562 kidney injury molecular-1, REG Iα: regenerating protein Iα, eGFR: estimated glomerular filtration rate,
563 CKD: chronic kidney disease; KDIGO: kidney disease improving global outcomes.
564 a: years, b: mmol/L, c: μmol /L, d: ng/mL, e: mg/L, f: pg/mL.

Figures

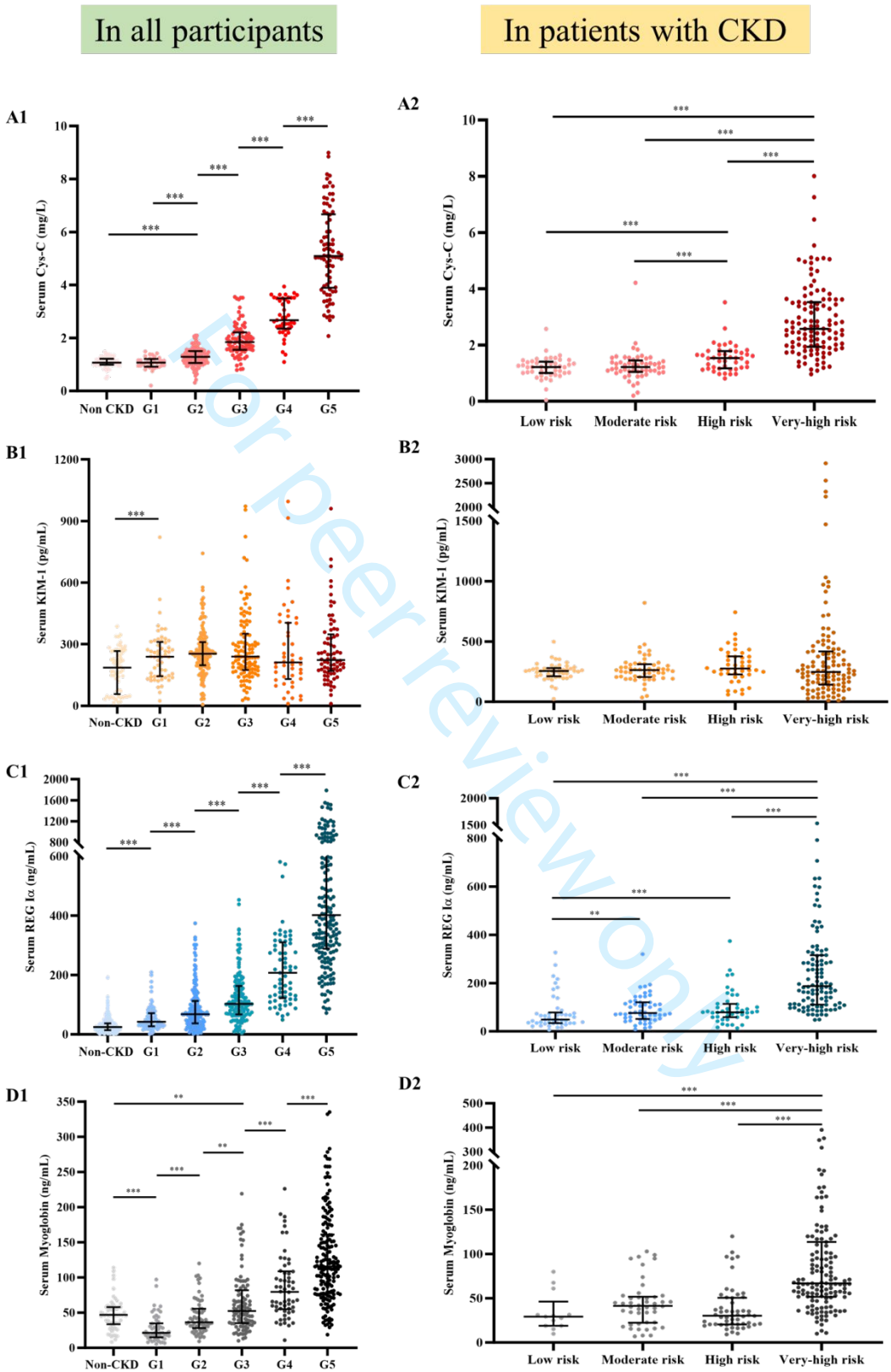


Figure 1. Distribution of serum Cys-C, serum KIM-1, serum REG Iα, and serum Myoglobin in different groups.

In all participants: the CKD groups were classified in accordance with eGFR levels as described in methods. A1: Distribution of serum Cys-C. The serum Cys-C level of non-CKD group was significantly lower compared to each of the G2, G3, G4, and G5 groups individually. B1: Distribution of serum KIM-1. C1: Distribution of serum REG Iα. The serum REG Iα level of non-CKD group was significantly lower compared to each of the G1, G2, G3, G4, and G5 groups individually. D1: Distribution of serum myoglobin. The serum myoglobin level of non-CKD was significantly lower compared to each of the G3, G4, and G5 groups individually.

In patients with CKD: the patients were classified in accordance with 2012 KDIGO risk stratification as described in methods. A2: Distribution of serum Cys-C. B2: Distribution of serum KIM-1. C2: Distribution of serum REG Iα. D2: Distribution of serum Myoglobin.

CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα. **: $P < 0.050$, ***: $P < 0.001$.

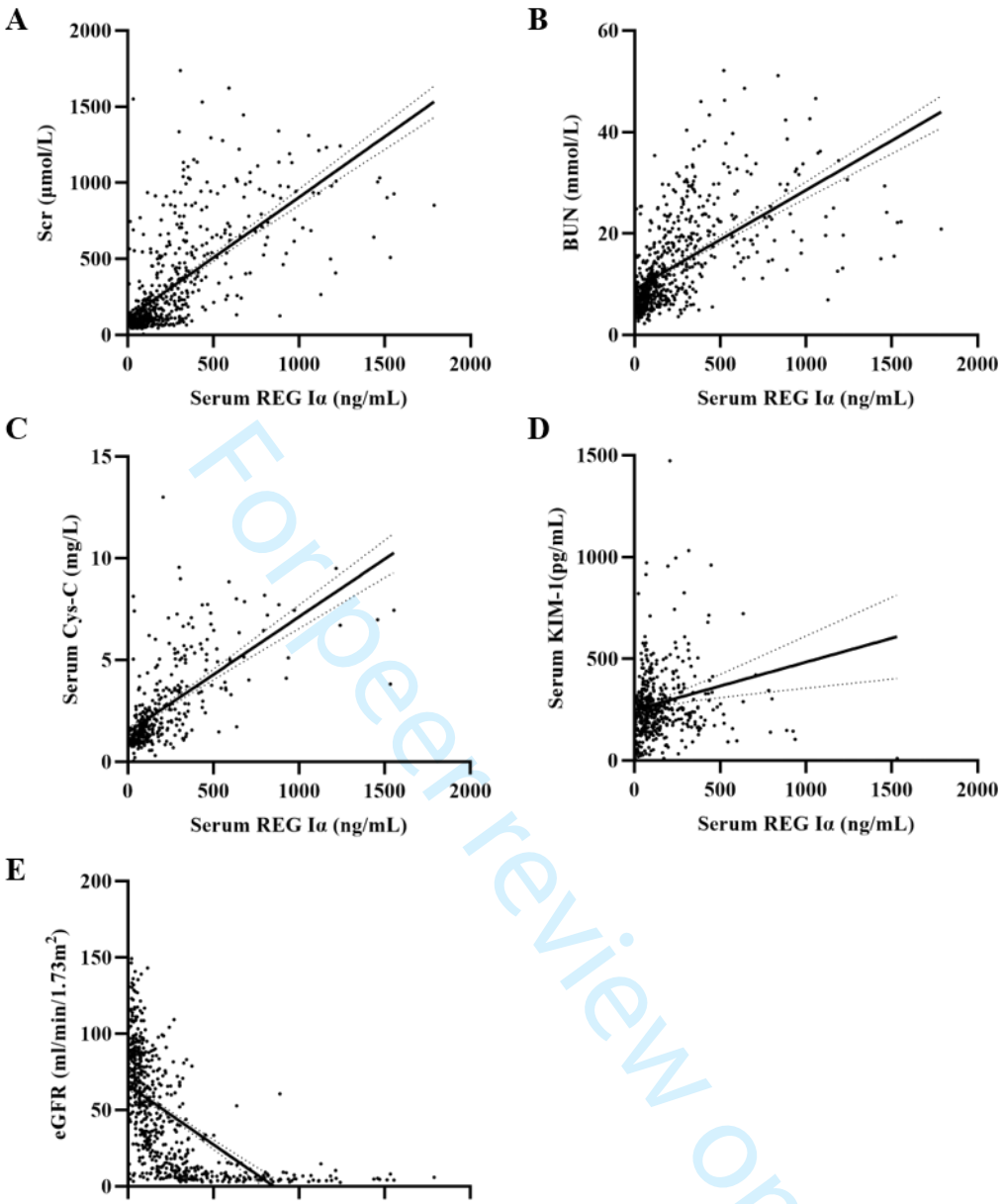


Figure 2. Correlations between serum REG Iα and different markers in all participants. A: correlation between serum REG Iα and Scr (r = 0.753, $P < 0.001$), B: correlation between serum REG Iα and BUN (r = 0.733, $P < 0.001$), C: correlation between serum REG Iα and serum Cys-C (r = 0.678, $P < 0.001$), D: correlation between serum REG Iα and serum KIM-1 (r = 0.217, $P < 0.001$), E: correlation between serum REG Iα and eGFR (r = -0.789, $P < 0.001$). Scr: serum creatinine, BUN: blood urea nitrogen, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, eGFR: estimated glomerular filtration rate, REG Iα: regenerating protein Iα.

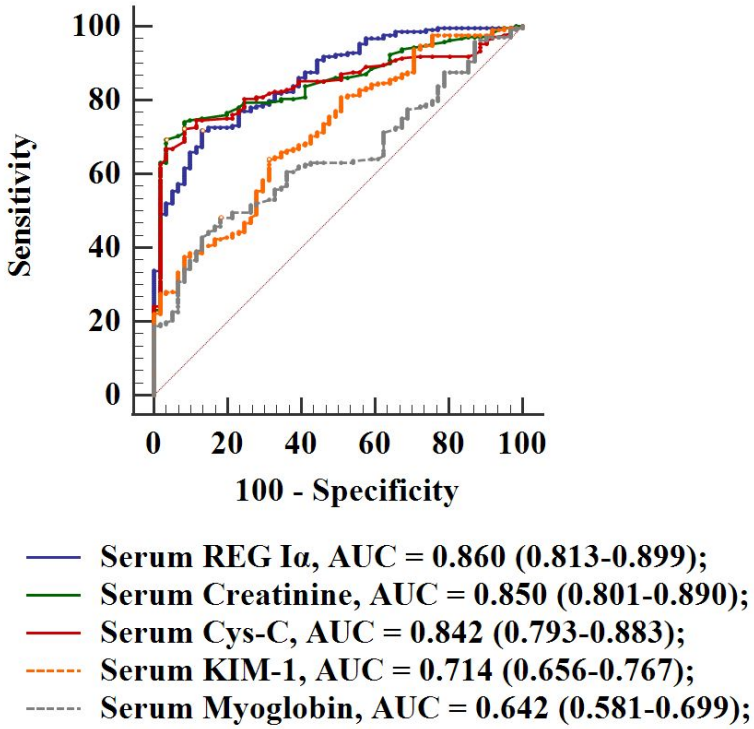


Figure 3. Ability of screening patients with CKD. The AUC of serum REG Iα was 0.860 (95% CI: 0.813-0.899), and that of serum Creatinine was 0.850 (95% CI: 0.801-0.890), while that of serum Cys-C was 0.842 (95% CI: 0.793-0.883). Serum KIM-1 had an AUC of 0.714 (95% CI: 0.656-0.767), and serum Myoglobin had an AUC of 0.642 (95% CI: 0.581-0.699). The AUC of serum REG Iα was similar to serum Creatinine and serum Cys-C ($P > 0.050$), and was significantly higher than serum KIM-1 and serum Myoglobin ($P < 0.001$). CKD: chronic kidney disease, AUC: area under the receiver operating characteristic curve, REG Iα: regenerating protein Iα, Cys-C: cystatin C, KIM-1: kidney injury molecular-1.

Supplementary material

Supplementary data Table 1. Correlations between serum REG Iα and different markers in all participants

Spearman Analyses (r)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age (1)	1.000													
Sex (2)	-0.060	1.000												
Diabetes (3)	0.367***	0.080**	1.000											
Hypertension (4)	0.262***	0.087**	0.236***	1.000										
CVD (5)	0.302***	0.024	0.125***	0.234***	1.000									
FBG (6)	0.258***	0.003	0.567***	0.050	0.062	1.000								
Scr (7)	0.037	0.294***	0.030	0.409***	0.098***	-0.159***	1.000							
BUN (8)	0.157***	0.170***	0.136***	0.442***	0.125***	-0.072**	0.845***	1.000						
UA (9)	-0.086**	0.267***	0.008	0.137***	0.036	-0.051	0.420***	0.379***	1.000					
eGFR (10)	-0.245***	-0.085**	-0.081**	-0.464***	-0.148***	0.123***	-0.927***	-0.856***	-0.328***	1.000				
Serum Cys-C (11)	-0.073	0.048	-0.237***	0.034***	0.079	-0.251***	0.811***	0.741***	0.336***	-0.826***	1.000			
Serum KIM-1 (12)	0.069	0.034	0.141***	0.078	0.031	0.105**	0.091**	0.121***	-0.076	-0.111**	0.111**	1.000		
Serum Myoglobin (13)	0.138***	0.117***	0.098**	0.229***	0.153***	0.024	0.648***	0.606***	0.210***	-0.661***	0.492***	0.066	1.000	

Serum REG Iα (14)	0.154***	0.059	0.073**	0.369***	0.099***	-0.134***	0.753***	0.733***	0.275***	-0.789***	0.678***	0.217***	0.565***	1.000

CVD: chronic kidney disease, FBG: fast blood glucose, Scr: serum creatinine, BUN: blood urea nitrogen, UA: serum uric acid, eGFR: estimated glomerular filtration rate, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα. **: $P < 0.050$, ***: $P < 0.001$.

Supplementary data Table 2. Distribution of serum Cys-C, serum KIM-1, serum REG Ⅰα and serum Myoglobin in KDIGO risk stratification.

KDIGO Risk Stratification Groups	**Low Risk (18.00%)**	**Moderate Risk (20.70%)**	**High Risk (17.20%)**	**Very-high Risk (44.10%)**
Serum Cys-C (mg/L)	1.23 (1.01-1.43)	1.22 (1.05-1.45)	1.54 (1.17-1.79)	2.57 (1.94-3.53)
Serum KIM-1 (pg/mL)	255.77 (211.33-279.86)	262.46 (205.21-312.24)	275.72 (227.08-377.36)	247.28 (142.72-417.99)
Serum Myoglobin (ng/mL)	29.30 (18.97-46.25)	41.50 (22.39-51.82)	30.25 (20.60-50.52)	67.00 (51.70-113.68)
Serum REG Ⅰα (ng/mL)	48.86 (34.18-78.28)	76.05 (51.09-120.54)	79.18 (58.64-113.76)	184.38 (108.81-314.71)

Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ⅰα: regenerating protein Ⅰα, KDIGO: kidney disease improving global outcomes.

The data were presented in quartiles.

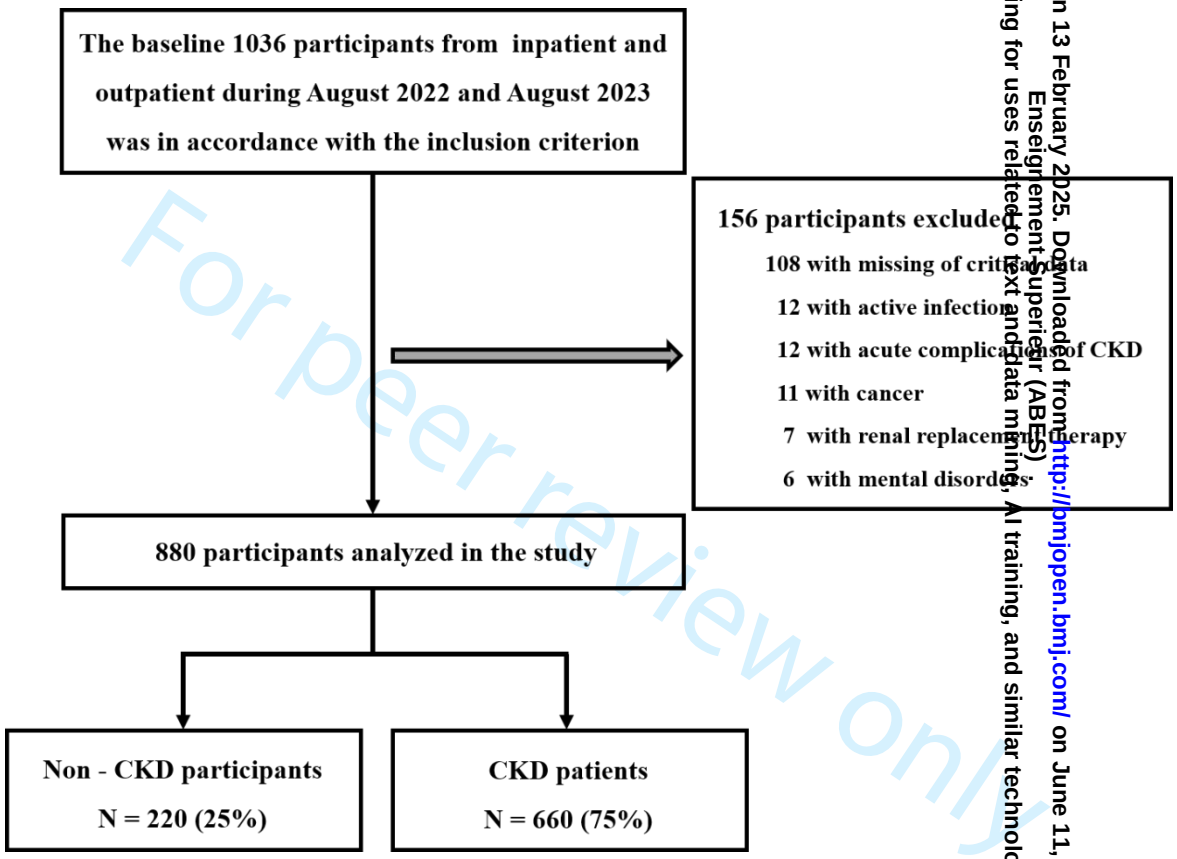
Supplementary data Table 3. Ability of different biomarkers to screen patients with CKD.

Variables	AUC (95% CI)	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Ability of screening patients with CKD in all participants						
Serum REG Iα (ng/mL)	0.860 (0.813-0.899)	70.82	71.63	86.89	91.30	46.85
Serum Creatinine (μmol/L)	0.850 (0.801-0.890)	88.00	69.23	96.72	97.33	47.90
Serum Cys-C (mg/L)	0.842 (0.793-0.883)	1.33	72.12	91.80	95.57	49.09
Serum KIM-1 (pg/mL)	0.714 (0.656-0.767) ***	232.98	63.94	68.85	88.93	35.09
Serum Myoglobin (ng/mL)	0.642 (0.581-0.699) ***	59.70	48.08	81.97	93.50	30.72
Ability of screening high and very-high risk patients according to KDIGO risk stratification in CKD participants						
Serum REG Iα (ng/mL)	0.769 (0.712-0.819)	76.05	82.80	62.63	77.38	69.32
Serum Cys-C (mg/L)	0.865 (0.817-0.904) ***	1.63	75.16	92.93	92.65	70.00
Serum KIM-1 (pg/mL)	0.528 (0.465-0.590) ***	327.10	36.94	87.88	81.69	46.49

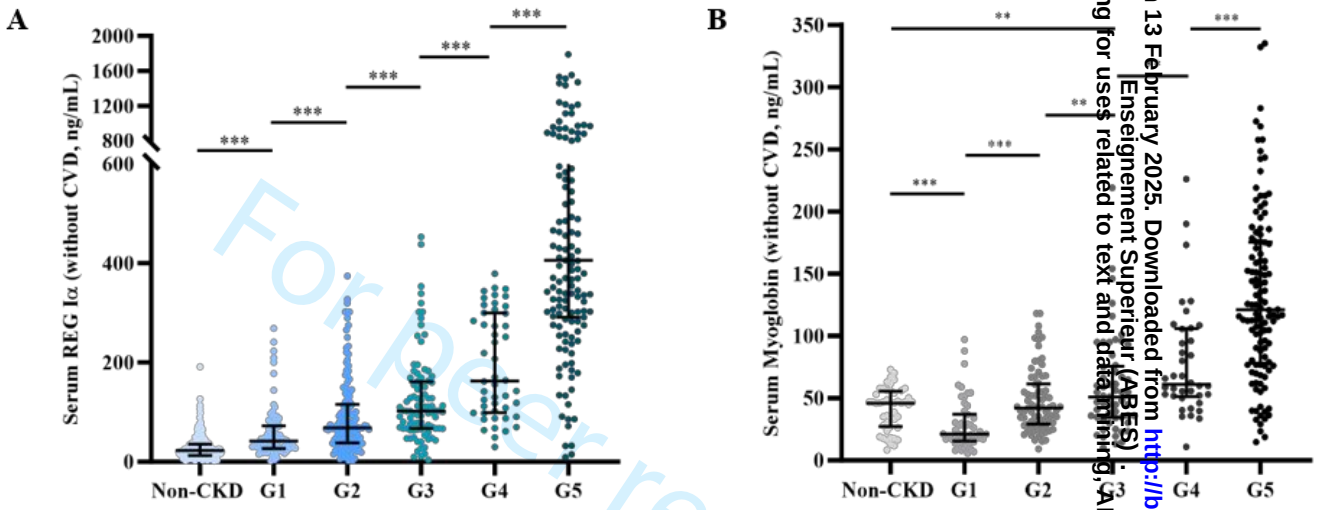
CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ⅰα: regenerating protein Ⅰα.

AUC: area under the receiver operating characteristic curve, PPV: positive predictive value, NPV: negative predictive value.

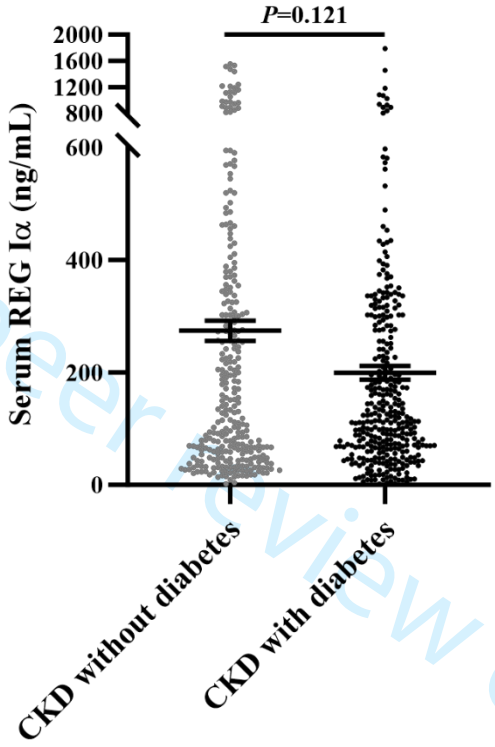
Comparing with serum REG Iα. ***: $P < 0.001$.



Supplementary data Figure 1. Flowchart of participant selection. In the 1036 inpatient and outpatient participants, 880 were eligible for inclusion. Based on the exclusion criterion, participants were excluded because of missing critical data (n=108), active infection (n=12), acute complications of CKD (n=12), cancer (n=11), renal replacement therapy (n=7), and mental disorders (n=6). CKD: chronic kidney disease.



Supplementary data Figure 2. Distribution of serum REG Iα and Myoglobin in non-CKD and different CKD groups, which participants without CVD. A: Distribution of serum REG Iα. The serum REG Iα level of non-CKD group was significantly lower than that of G1, G2, G3, G4, and G5 groups individually. B: Distribution of serum myoglobin. The serum myoglobin level of non-CKD was significantly lower than that of G3, G4, and G5 groups individually. CKD: chronic kidney disease, CVD: cardiovascular disease, REG Iα: regenerating protein Iα. **: $P < 0.050$, ***: $P < 0.001$.



Supplementary data Figure 3. Levels of serum REG Iα in CKD patients with and without T2DM. The median with interquartile range of serum REG Iα was 144.37 (54.71-357.59) ng/ml in CKD patients without T2DM, and 116.57 (65.86-276.34) ng/ml. REG Iα: regenerating protein Iα, T2DM: type 2 diabetes mellitus.

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

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Primary Subject Heading:	Renal medicine
Secondary Subject Heading:	Renal medicine

Keywords:	Chronic Disease, Cross-Sectional Studies, Nephrology < INTERNAL MEDICINE, Chronic renal failure < NEPHROLOGY

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

Author

Nan Huang[1, 2, 3#], Xiangyun Zhu[1, 3#], Zhiyi Shu[1, 3#], Sheng Chen[1, 3], Xiaodong Wu[1, 3], Hui Wang[1, 3], Xi Huang[1], Xiuxiu Hu[4], Jinfang Sun[5], Pingsheng Chen[4], Rolf Graf[6], Jianling Bai[7*], Bin Wang[8*], Ling Li[1, 2, 3*]

Address

1. Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China.

2. Key Laboratory of Environmental Medicine Engineering of Ministry of Education, Southeast University, Nanjing, Jiangsu, 210009, China.

3.Pancreatic Research Institute, Southeast University, Nanjing, Jiangsu 210009, China.

4. Department of Pathology, School of Medicine, Southeast University, Nanjing 210009, China.

5. Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu 210009, China.

6. Department of Visceral and Transplantation Surgery, University Hospital of Zurich, Zurich, 8091, Switzerland.

7. Department of Biostatistics, School of Public Heath, Nanjing Medical University, Nanjing, Jiangsu, 211166, China.

8. Department of Nephrology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China.

Correspondence

# These authors have contributed equally to this article.

* Address correspondence to:

1. Ling Li*: M.D.

Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China. (e-mail: lingli@seu.edu.cn);

2. Bin Wang*: Ph.D.

Department of Nephrology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China. (email: wangbinhewei@126.com);

3. Jianling Bai*: Ph.D.

Department of Biostatistics, School of Public Heath, Nanjing Medical University, Nanjing, Jiangsu, 211166, China. (email: baijianling@njmu.edu.cn);

Abstract

Objectives

This study conducted to demonstrate the relationship between levels of serum regenerating protein Iα (REG Iα) and estimated glomerular filtration rate (eGFR) and to explore the efficiency of REG Iα in chronic kidney disease (CKD) detection.

Design

A cross-sectional study.

Setting

Conducted in eastern China between August 2022 and August 2023.

Participants

880 participants which age older than 18 years were enrolled in this study, with 220 non-CKD participants (111 in male, 50.45%) and 660 patients with CKD (366 in male, 55.45%). The diagnostic criteria of CKD were in accordance with the kidney disease: improving global outcomes (KDIGO) guidelines in 2012. The exclusion criteria were included with involvement in other trials, acute kidney injury, patients with end-stage of kidney disease who are undergoing renal replacement therapy, pregnancy, active infection, gastrointestinal or pancreatic inflammation, history of gastrointestinal or pancreatic resections, cancer, mental disorders.

Results

In CKD group, the levels of serum REG Iα (125.54 [60.28-303.39] ng/mL) were significantly higher compared to those in non-CKD group (24.62 [14.09-37.32] ng/mL, $P < 0.001$). Serum REG Iα exhibited a positive relationship with serum creatinine (Scr), cystatin C (Cys-C), and kidney injury molecular-1(KIM-1), while showed a negative relationship with eGFR. 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], $P = 0.001$). Furthermore, levels of serum REG Iα were found to gradually increase with the decline of kidney function related to eGFR ($P < 0.001$). Serum REG Iα was recognized to play a role in screening CKD patients, with an area under the receiver operating

characteristic curves (AUC) of 0.860 (0.813-0.899), providing a sensitivity of 71.63%, a specificity of 86.89%, a positive predictive value of (PPV) 94.30%, and a negative predictive value (NPV) of 46.85%. Additionally, serum REG Iα had an AUC of 0.769 (0.712-0.819) in screening patients at high and very-high risk for CKD according KDIGO risk stratification, exhibiting significantly higher sensitivity compared to serum Cys-C and KIM-1 (82.80% vs 75.16% and 36.94%).

Conclusions

This study provided evidence that levels of serum REG Iα were notably elevated in patients with CKD and may be closely related to kidney function. The findings indicate potential utility for REG Iα as a biomarker in CKD.

Clinical trial registration

The study was approved by the ethics committee of Zhongda Hospital (2022ZDSYLL204-P01) and was in compliance with the Helsinki Declaration. The study had a clinical study registration number of ChiCTR2300072247.

Data availability statement

The data underlying this article is available from the corresponding author under reasonable request.

Patient and public involvement statement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

Strengths and limitations of this study

- We utilized different logistic regression analyses to adjust for various confounding factors and investigate the relationship between serum REG Iα and kidney function.
- This study was the first time to apply KDIGO risk stratification in CKD population, analyzing the potential association between serum REG Iα and the risk of CKD progression.
- We applied the DeLong test to statistically analyze the differences in AUC values

among the biomarkers of CKD, assessing their diagnostic abilities.

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Competing interests' statement

The authors declare that they have no competing interests.

Keywords

Regenerating protein Iα, Chronic kidney disease, Biomarker, Kidney function, Risk stratification;

Abbreviations

List of abbreviations	
Regenerating protein Iα	REG Iα
Diabetic kidney disease	DKD
Chronic kidney disease	CKD
Receiver operating characteristic curves	ROC
Cystatin C	Cys-C
Kidney injury molecular-1	KIM-1
Area under the ROC	AUC
End stage kidney disease	ESKD
Serum creatinine	Scr
Blood urea nitrogen	BUN
Estimate glomerular filtration rate	eGFR
Urine albuminuria creatine ratio	UACR
Kidney disease improving global outcomes	KDIGO
Pancreatic stone protein	PSP
Chronic kidney disease epidemiology collaboration	CKD-EPI
Unilateral ureteral obstruction	UUO
Uric acid	UA
Fast blood glucose	FBG
Cardiovascular disease	CVD
Standard deviation	SD

Introduction

Chronic kidney disease (CKD) has a broad range of underlying etiologies and variable progression rates, [1,2] and may become the fifth leading cause of death worldwide by 2040.[3] The endpoint of CKD is end stage kidney disease (ESKD) in which 90% of kidney function reduced, making it impossible to sustain life over the long term.[2] The high prevalence, low detection rate, severe outcomes, and substantial medical costs of CKD make it a significant global health concern.[4] Early prevention, detection, and treatment can lead to better outcomes and prevent ESKD progression.

Biomarkers such as serum creatinine (Scr), blood urea nitrogen (BUN), estimate glomerular filtration rate (eGFR), and urine albuminuria creatine ratio (UACR) are routinely used to assess the severity of CKD.[5-8] In 2012, Kidney Disease: Improving Global Outcomes (KDIGO) has taken the eGFR and UACR to create risk stratification strategies, assisting in the tailored management of CKD and guiding treatment for those at a higher risk of progression.[9] New biomarkers like, cystatin C (Cys-C), kidney injury molecular-1 (KIM-1), and β2-microglobuline have shown great application potential in enhancing the precision of early-stage CKD screening, either independently or in conjunction.[2,3,10,11] However, most biomarkers failed to match clinical expectations.[12-15] Few biomarkers can both detect and assess risk stratification effectively in patients with CKD. Thus, discovering a biomarker can screen kidney function decline and evaluate CKD progression risk is crucial.

Regenerating protein Iα (REG Iα) is a 16 kDa protein primarily secreted by the pancreas and intestine,[16] also known as pancreatic stone protein (PSP).[17] The involvement of REG Iα plays a role in the processes of cellular proliferation and regeneration.[18,19] Recent researchers have identified the presence of REG Iα in patients with various kidney diseases.[20,21] Our previous studies also have indicated that serum levels of REG Iα elevate in patients with diabetic kidney disease (DKD), which are concordant with the finding of H. Sobajima.[22-24] These evidences indicate that REG Iα may serve as a biomarker of kidney insufficiency.

In this study, we analyzed the association between serum REG Iα levels and kidney

function, and further assessed its potential as a screening tool for CKD and as a biomarker of kidney function.

Methods

Study subjects

The participants were enrolled from Zhongda Hospital between August 2022 and August 2023. The study was approved by the ethics committee (2022ZDSYLL204-P01), with a clinical study registration of ChiCTR2300072247. Informed consent was acquired from all participants.

The inclusion criteria were as follows: (1) non-CKD participant: age older than 18 years; (2) CKD patients: age older than 18 years and diagnosed with CKD in accordance with the guidelines in 2012.[1] The exclusion criteria were as follows: (1) enrolled in another trial; (2) acute kidney injury; (3) patients with ESKD who are undergoing renal replacement therapy; (4) pregnancy; (5) active infection; (6) acute or chronic inflammation of the gastrointestinal system and pancreas; (7) history of gastrointestinal or pancreatic resections; (8) cancer; (9) mental disorders.

eGFR was calculated in accordance with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[25] CKD groups was classified using the method proposed by the U.S. National Kidney Foundation. The risk stratification of CKD progression was defined in accordance with KDIGO risk stratification guideline.[26,27] The subgroup analyses was carried out in patients with CKD to explore the relationship between serum REG Iα and different degrees of kidney function impairment.

Data collection and quality assessment

Demographics information was collected at baseline through questionnaires. All participants have undergone 12-h fasting and taken about 3mL of peripheral blood sample. The blood samples were centrifuged directly for 15 min at a rotating speed of 3,500 rpm, and the upper serum was sucked up within 6-h. The serum was immediately frozen in refrigerator (-80℃). Clinical biochemical parameters were extracted from the clinical laboratory of Hospital, such as Scr, BUN, uric acid (UA), Cys-C, Myoglobin, UACR, and fast blood glucose (FBG). The laboratory implements internal and external

quality control procedures directed by a Chinese Quality Control Laboratory. The serum REG Iα levels were determined by a double antibody sandwich ELISA, as previously described.[28] Serum KIM-1 was detected by an ELISA kit (KE00136) from Proteintech.

Statistical analysis

Statistical analyses were conducted using SPSS 20.0, Med-Calc, and GraphPad Prism 8.0. Continuous data with normal distribution were summarized as mean ± standard deviation (SD), otherwise as median with interquartile range. For categorical variables, the frequency with a percentage of each category was calculated. 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. 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 study assessed evaluate accuracy using sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and accuracy, and applied the DeLong test to statistically analyze AUC differences between receiver operating characteristic curves. *P* value of <0.050 using two-tailed tests was considered statistically significant.

Results

Baseline characteristics of the study population

Overall, 880 participants were finally enrolled, with 220 non-CKD participants and 660 patients with CKD (Supplementary data Figure 1). Differences in age, complication diseases (diabetes and hypertension), kidney function biomarkers (Scr, BUN, UA, eGFR, Cys-C, and KIM-1), and serum myoglobin were observed between patients with CKD and non-CKD participants (all $P < 0.001$, Table 1). Serum REG Iα levels were notably higher in patients with CKD (125.54 [60.28-303.39] ng/mL), than in non-CKD participants (24.62[14.09-37.32] ng/mL, $P < 0.001$, Table 1). Serum Cys-C, REG Iα, and myoglobin showed increasing trends as eGFR levels decreased (Figure 1: A1, C1,

and D1). A significant difference in serum KIM-1 was found between the non-CKD group and CKD groups (G2 to G5, $P < 0.010$, Figure 1: B1). Two reanalyzes balanced the effects of CVD on the distribution of serum REG Iα and myoglobin (supplementary data Figure 2), and the effects of diabetes on patients with CKD (Supplementary data Figure 3).

Relationship between serum REG Iα and kidney function

This study explored the relationship between serum REG Iα levels and kidney function biomarkers. In figure 2, Spearman correlation analyses demonstrated that serum REG Iα was positively correlated with Scr (r = 0.753, $P < 0.001$), BUN (r = 0.733, $P < 0.001$), serum Cys-C (r = 0.678, $P < 0.001$), and serum KIM-1 (r = 0.217, $P < 0.001$), and negatively correlated with eGFR (r = -0.789, $P < 0.001$). The comprehensive correlations between serum REG Iα and various markers were presented in supplementary data Table 1. In ordinal logistic regression analysis carried out in all participants, eGFR values were used as a grade-dependent variable in the model. The results showed that serum REG Iα/100 levels was markedly associated with eGFR (OR=1.737 95% CI: 1.263-2.388, $P = 0.001$, Table 2).

Subgroup analysis in patients with CKD

To investigate the relationship between serum REG Iα and the risk of CKD progression, 256 CKD patients were included in the sub-research and were classified by eGFR and UACR levels in accordance with KDIGO risk stratification guideline.[1] The patients were divided into four groups to assess the progression and prognostic, namely, low risk (18.00%), moderate risk (20.70%), high risk (17.20%) and very-high risk (44.10%, supplementary data Table 2). We found that levels of serum Cys-C significantly increase to 2.57 (1.94, 3.53) mg/L in the very-high risk group ($P < 0.001$, Figure 1: A2). In the same trends, the levels of serum REG Iα significantly increase to 184.38 (108.81-314.71) ng/mL in the very-high risk group ($P < 0.001$, Figure 1: C2). The very-high risk group had the highest serum myoglobin level among the four groups ($P < 0.001$, Figure 1: D2). The multiple logistic regression analysis carried out in patients with CKD, demonstrated that serum REG Iα/100 was an independent influencing factor for patients

with CKD who are at high and very-high risk (OR=1.799, 95% CI: 1.088-2.975, P = 0.022, Table 2).

Ability of serum REG Iα in screening patients with kidney dysfunction

In evaluating the potential application of serum REG Iα as a screening tool for patients with CKD and its ability to differentiate the risk stratification, we performed receiver operating characteristic curve (ROC) analyses (Figure 3 and supplementary data Table 3). The AUC for serum REG Iα to detect patients with CKD was 0.860 (95% CI: 0.813-0.899) as well as serum creatinine (0.850, 95% CI: 0.801-0.890) and Cys-C (0.842, 95% CI: 0.793-0.883), with a cutoff value of 70.82 ng/mL, sensitivity of 71.63%, specificity of 86.89%, positive predictive value (PPV) of 94.30%, and negative predictive value (NPV) of 46.85%. By contrast, serum KIM-1 had a lower AUC than serum REG Iα, measuring 0.714 (95% CI: 0.656-0.767, $P < 0.001$). Serum myoglobin had the lowest AUC among the five biomarkers, measuring 0.642 (95% CI: 0.581-0.699, $P < 0.001$). Furthermore, we analyzed the ability of serum Cys-C, KIM-1, and REG Iα in distinguishing high and very-high risk patients with CKD in accordance with the KDIGO risk stratification. As shown in supplementary data Table 3, serum Cys-C had an AUC of 0.865 (95% CI: 0.817-0.904, $P < 0.010$), which was the highest AUC among the three markers. Serum REG Iα had an AUC of 0.769 (95% CI: 0.712-0.819), showing better performance than serum KIM-1 ($P < 0.010$). The cutoff value of serum REG Iα was 76.05 ng/mL, and the sensitivity, specificity, PPV, and NPV were 82.80%, 62.63%, 77.38%, and 69.32%, respectively. Notably, serum REG Iα had a significantly higher sensitivity than serum Cys-C and KIM-1.

Discussion

This study was the first time to investigate the whole CKD population, and confirm the upregulation of serum REG Iα in patients with CKD. Initially, the serum levels of REG Iα showed a gradually increasing trend with the decrease of eGFR. Second, serum REG Iα was negatively correlated with eGFR and positively correlated with Scr, BUN, serum Cys-C, and serum KIM-1 levels. Third, serum REG Iα was an independent risk factor for high and very-high risk patients in accordance with the KDIGO risk stratification.

Finally, serum REG Iα played an important role in screening patients with CKD and detecting high and very-high risk patients in CKD.

REG Iα was a low molecular weight (16 kDa) protein initially discovered in pancreas, where it exhibited a remarkable ability to inhibit the formation of calcium carbonate stones in pancreatic ducts.[29] Hence, REG Iα was also called pancreatic stone protein (PSP) at the time.[30] Immunocytochemical analyses indicated that REG Iα protein was overexpressed in impaired kidney, with predominant localization observed in proximal tubules and thick ascending limbs of Henle's loops.[20] Then, some studies declared that urine PSP (REG Iα) and *REG Iα* gene might indicate kidney tubular dysfunction and potentially serve as an early biomarker of DKD detection and progression.[22,31,32] Our previous studies provided compelling evidence, that serum REG Iα levels were notably elevated in patients with type 2 diabetes mellitus who had kidney dysfunction. And we also observed that pregnant women with early renal dysfunction exhibited similar elevations in serum REG Iα levels.[24,33] Notably, the serum REG Iα levels remained significantly elevated when the scope of this study was expanded to the entire CKD population.

We incorporated myoglobin (17.6 kDa)[34] to investigate whether the elevated level of serum REG Iα represents a universal phenomenon of accumulation in CKD. The myoglobin was identified as a biomarker for acute myocardial ischemia and rhabdomyolysis.[35] Some studies indicated that serum myoglobin levels increase in kidney impairment. This accumulation was primarily attributed to slower clearance in CKD.[36] We showed that serum levels of myoglobin have no differences between non-CKD participants and patients with early stage of CKD (group G1and group G2). And there was a gradual increase in patients with medium to end-stages of CKD (group G3 to group G5). The possible reason was as follows: in the early stages of kidney injury, compensatory glomerular hypertrophy with hyperfiltration state was known to exist. This mechanism allowed some low molecular weight protein to be filtered through the glomerular basement membrane, resulting in no discernible difference during the early stage of CKD (eGFR ≥ 60 mL/min per 1.73 m^2), and even appearing as a transient

decrease. With progression to medium to end-stages of CKD (eGFR < 60 mL/min per 1.73 m^2), the glomerular basement membrane thickened and led to significant decline in glomerular filtration function, ultimately causing proteins accumulation in serum. [37] The serum levels of REG Iα showed a gradual increase in medium to end-stages of CKD, which was similar to the rise observed in serum myoglobin levels. However, it exhibited a significant increase in the early stage of CKD, which was different with serum myoglobin. This suggested that, in addition to the sensitive accumulation of serum REG Iα as a low molecular weight protein, there might be a specific production when it comes to kidney impairment.

The etiology underlying the upregulation of REG Iα production in patients with CKD remains elusive. Two potential mechanisms are considered as follows. First, REG Iα resists apoptosis and promotes cell proliferation in different inflammation situations.[38-42] Studies have shown that cytokines such as IL-6 can increase the proliferation of REG Iα, which is involved in cell regeneration and repair.[42,43] In CKD, the occurrence and progression of the disease are closely related to the expression of multiple inflammatory cytokines, including IL-1, IL-6, and TNF-α. [37,44,45] Under the stimulation of chronic inflammation, different types of renal cells secrete REG Iα locally to participate in kidney anti-apoptosis and proliferation and against kidney fibrosis in the development of CKD. The secreted REG Iα enters the circulation through reabsorption by kidney tubules, resulting in a significant increase in serum REG Iα levels. Thus, REG Iα might serve as an inflammatory factor involved in kidney diseases. Second, REG Iα is primarily synthesized in the pancreas and released into the circulation.[18,46] A hypothesis suggests that a cross-talk mechanism exists between the pancreas and kidney when renal damage occurs. This interaction may prompt the pancreas to produce more REG Iα in response to kidney injury, resulting the elevation in serum. Although the exact cause of REG Iα upregulation remains unclear, these two proposed mechanisms provided potential insights into the relationship between REG Iα and CKD.

At present, the assessment of CKD generally focused on glomerular filtration capacity, which is characterized by Scr, UACR, and eGFR.[2] The stabilities of these factors are

compromised by age, dietary intake, and physical activity. In general, the performance of eGFR is poor for populations outside of North America, Australia, and Europe, and accuracy decreases at the extremes in the distribution of age, and body composition.[7] Therefore, many new biomarkers of kidney impairment have been discovered. Cys-C is produced by all nucleated cells at a steady rate and freely filtered by the kidney.[47] The accumulation of its levels is observed in case of glomerular filtration dysfunction, with limited impact and strong stability.[48] Eventually, serum Cys-C is incorporated into the eGFR equation as a reliable and sensitive indicator of kidney function. The sensitivity of serum Cys-C is heightened in the early stages of CKD compared to creatinine.[49,50] Another biomarker is KIM-1,[51,52] which is secreted following kidney proximal tubular injury, and has emerged as a sensitive and specific biomarker of acute kidney injury and CKD progression.[53-56] In this study, serum REG Iα strongly correlated with serum creatinine and Cys-C, and had a similar performance to serum creatinine and Cys-C in detecting patients with CKD. The serum levels of REG Iα were more likely related to glomerular filtration capacity according to the correlation analyses, although the mechanism of its production and degradation or excretion in CKD remained unclear. We indicated that serum REG Iα might be more sensitive than serum creatinine and Cys-C in detecting ultra-early stage of kidney dysfunction. Similar phenomenon was observed in distribution analyses in KDIGO risk stratification. The KDIGO risk classification was widely used in evaluating the progression and prognosis risk in patients with CKD.[57-60] Serum levels of REG Iα gradually elevated with higher KDIGO risk stratification categories. And it emerged as an independent risk factor for patients with CKD categorized as high and very-high risk. Our findings provided a new insight that serum REG Iα performed better than serum KIM-1 in screening patients with CKD and detecting high and very-high risk patients with CKD. Moreover, serum REG Iα displayed a highest sensitivity in identifying high and very-high risk patients with CKD. These results highlight the potential application of serum REG Iα as a valuable biomarker in the screening of patients with CKD and the assessment of CKD risk.

We observed that patients with CKD were older than non-CKD participants, and they had higher rates of diabetes and hypertension. This finding was consistent with the typical etiology of CKD. Globally, diabetes and hypertension are recognized as the primary causes of CKD.[2] Diabetes accounts for 30-50% of all CKD cases and affects approximately 285 million adults worldwide. A consistent finding in observational studies shows that the increased risk of developing is associated with blood pressure control. These findings were consistent with the expected proportion of CKD etiology in our study.

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. Forth, to address residual confounding, future studies should incorporate additional covariates such as drug use and different causes of CKD and conduct sensitivity analyses to assess potential biases. Moreover, to enhance causality and generalizability, longitudinal designs with larger sample sizes and diverse populations should be considered in forthcoming research.

Conclusion

This study provided compelling evidence that serum REG Iα was significantly upregulated in the patients with CKD and strongly associated with kidney function. Serum REG Iα emerged as a significant biomarker for detecting kidney function decline.

Contributorship statement

Professor Ling Li acted as guarantor. Ling Li conceptualized the study; Bin Wang reviewed and edited the manuscript; Nan Huang and Xiangyun Zhu were responsible for the data analysis and wrote the original draft. Nan Huang, Sheng Chen, Xiaodong Wu, Hui Wang, Zhiyi Shu and Xi Huang were responsible for inclusion of population, collection of samples and data, and the experiments. Jianling Bai and Jinfang Sun were responsible for guidance on data analysis. Pingsheng Chen and Xiuxiu Hu were responsible for analysis of kidney biopsy tissues from CKD patients. Rolf Graf provided the excellent technical support for this study.

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Tables

Table 1. Clinical characteristics of study population at baseline examination.

CKD: chronic kidney disease; The data were presented in quartiles.

Table 2. The logistic regression analyses showing the relationship between variables and kidney function.

#: The ordinal multiple logistic regression showing variables independently associated with eGFR levels in all participants. *: The multivariate logistic regression analysis identified the independent influencing factors for high and very-high risk patients with CKD in accordance with KDIGO risk stratification. The two logistic regression analyses have been adjusted the sex, diabetes, hypertension, and FBG.

FBG: fast blood glucose, BUN: blood urea nitrogen, UA: serum uric acid, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα, eGFR: estimated glomerular filtration rate, CKD: chronic kidney disease; KDIGO: kidney disease improving global outcomes.

a: years, b: mmol/L, c: μmol /L, d: ng/mL, e: mg/L, f: pg/mL.

Figures

Figure 1. Distribution of serum Cys-C, serum KIM-1, serum REG Iα, and serum Myoglobin in different groups.

A1, B1, C1, and D1 are in all participants: the CKD groups were classified in accordance with eGFR levels as described in methods. A1: Distribution of serum Cys-C. B1: Distribution of serum KIM-1. C1: Distribution of serum REG Iα. D1: Distribution of serum myoglobin. A2, B2, C2, and D2 are in patients with CKD: the patients were classified in accordance with 2012 KDIGO risk stratification as described in methods. A2: Distribution of serum Cys-C. B2: Distribution of serum KIM-1. C2: Distribution of serum REG Iα. D2: Distribution of serum Myoglobin.

CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

Figure 2. Correlations between serum REG Iα and different markers in all participants.

A: correlation between serum REG Iα and Scr ($r = 0.753$, $P < 0.001$), B: correlation between serum

REG Iα and BUN (r = 0.733, $P < 0.001$), C: correlation between serum REG Iα and serum Cys-C (r = 0.678, $P < 0.001$), D: correlation between serum REG Iα and serum KIM-1 (r = 0.217, $P < 0.001$), E: correlation between serum REG Iα and eGFR (r = -0.789, $P < 0.001$). Scr: serum creatinine, BUN: blood urea nitrogen, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, eGFR: estimated glomerular filtration rate, REG Iα: regenerating protein Iα.

Figure 3. Ability of screening patients with CKD.

The AUC of serum REG Iα was 0.860 (95% CI: 0.813-0.899), and that of serum Creatinine was 0.850 (95% CI: 0.801-0.890), while that of serum Cys-C was 0.842 (95% CI: 0.793-0.883). Serum KIM-1 had an AUC of 0.714 (95% CI: 0.656-0.767), and serum Myoglobin had an AUC of 0.642 (95% CI: 0.581-0.699). The AUC of serum REG Iα was similar to serum Creatinine and serum Cys-C ($P > 0.050$), and was significantly higher than serum KIM-1 and serum Myoglobin ($P < 0.001$). CKD: chronic kidney disease, AUC: area under the receiver operating characteristic curve, REG Iα: regenerating protein Iα, Cys-C: cystatin C, KIM-1: kidney injury molecular-1.

Supplementary material

Supplementary data Table 1. Correlations between serum REG Iα and different markers in all participants.

CVD: chronic kidney disease, FBG: fast blood glucose, Scr: serum creatinine, BUN: blood urea nitrogen, UA: serum uric acid, eGFR: estimated glomerular filtration rate, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα. **: $P < 0.050$, ***: $P < 0.001$.

Supplementary data Table 2. Distribution of serum Cys-C, serum KIM-1, serum REG Ⅰα and serum Myoglobin in KDIGO risk stratification.

Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ⅰα: regenerating protein Ⅰα, KDIGO: kidney disease improving global outcomes. The data were presented in quartiles.

Supplementary data Table 3. Ability of different biomarkers to screen patients with CKD.

CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ⅰα: regenerating protein Ⅰα. AUC: area under the receiver operating characteristic curve, PPV: positive predictive value, NPV: negative predictive value.

Comparing with serum REG Iα. ***: $P < 0.001$.

Supplementary data Figure 1. Flowchart of participant selection.

In the 1036 inpatient and outpatient participants, 880 were eligible for inclusion. Based on the exclusion criterion, participants were excluded because of missing critical data (n=108), active infection (n=12), acute complications of CKD (n=12), cancer (n=11), renal replacement therapy (n=7), and mental disorders (n=6). CKD: chronic kidney disease.

Supplementary data Figure 2. Distribution of serum REG Iα and Myoglobin in non-CKD and different CKD groups, which participants without CVD.

A: Distribution of serum REG Iα. The serum REG Iα level of non-CKD group was significantly lower than that of G2, G3, G4, and G5 groups individually. B: Distribution of serum myoglobin. The serum myoglobin level of non-CKD was significantly lower than that of G4 and G5 groups individually. CKD: chronic kidney disease, CVD: cardiovascular disease, REG Iα: regenerating protein Iα. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.

Supplementary data Figure 3. Levels of serum REG Iα in CKD patients with and without T2DM.

The median with interquartile range of serum REG Iα was 144.37 (54.71-357.59) ng/ml in CKD patients without T2DM, and 116.57 (65.86-276.34) ng/ml. REG Iα: regenerating protein Iα, T2DM: type 2 diabetes mellitus.

Tables

Table 1. Clinical characteristics of study population at baseline examination.

	Non-CKD Participants	CKD Patients	*P* Value
Number	220	660	-
DEMOGRAPHICS			
Age (years)	53 (40-62)	62 (50-72)	< 0.001
Sex (male, %)	111 (50.45)	366 (55.45)	0.212
Smoking (yes, %)	41 (18.64)	142 (21.52)	0.141
Drinking (yes, %)	30 (13.64)	84 (12.73)	0.823
COMPLICATION DISEASES			
Diabetes (yes, %)	75 (34.09)	350 (53.03)	< 0.001
Hypertension (yes, %)	65 (29.55)	501 (75.91)	< 0.001
Cardiovascular disease (CVD, yes, %)	44 (20.00)	149 (22.58)	0.453
LABORATORY MEASUREMENTS			
Fast blood glucose (FBG, mmol/L)	5.45 (4.99-6.67)	5.34 (4.57-7.11)	0.008
Serum creatinine (Scr, μmol/L)	64.00 (55.25-76.00)	126.00 (83.00-418.50)	< 0.001
Blood urea nitrogen (BUN, mmol/L)	5.20 (4.30-6.28)	10.30 (7.00-18.60)	< 0.001
Serum uric acid (UA, μmol/L)	301.00 (256.00-365.25)	354.00 (290.00-443.00)	< 0.001
Estimated glomerular filtration rate (eGFR, mL/min per 1.73 m^2)	102.37 (95.73-112.36)	44.03 (10.96-77.09)	< 0.001
Serum cystatin C (Cys-C, mg/L)	1.10 (0.99-1.24)	1.79 (1.23-3.50)	< 0.001
Serum kidney injury molecular-1 (KIM-1, pg/mL)	186.28 (57.22-266.88)	247.72 (175.10-334.13)	< 0.001
Serum Myoglobin (ng/mL)	46.81 (33.49-58.00)	64.04 (35.96-112.51)	< 0.001
Serum regenerating protein Iα (REG Iα, ng/mL)	24.62 (14.09-37.32)	125.54 (60.28-303.39)	< 0.001

CKD: chronic kidney disease; The data were presented in quartiles.

Table 2. The logistic regression analyses showing the relationship between variables and kidney function.

	Ordinal Logistic Regression[#]		Multivariate Logistic Regression*	
	***P* Value**	**OR (95% CI)**	***P* Value**	**OR (95% CI)**
Age[a]	0.050	1.020 (1.001-1.041)	0.009	0.966 (0.942-0.992)
BUN[b]	< 0.001	1.266 (1.165-1.376)	< 0.001	1.440 (1.216-1.706)
UA[c]	0.337	1.001 (0.999-1.004)	0.085	1.003 (1.000-1.007)
Serum Myoglobin[d]	0.165	1.005 (0.998-1.013)	N/A	N/A
Serum Cys-C[e]	< 0.001	6.784 (4.016-11.460)	0.071	1.853 (0.949-3.620)
Serum KIM-1/100[f]	0.133	1.069 (0.980-1.167)	0.122	1.243 (0.943-1.639)
Serum REG Iα/100[d]	0.001	1.737 (1.263-2.388)	0.022	1.799 (1.088-2.975)

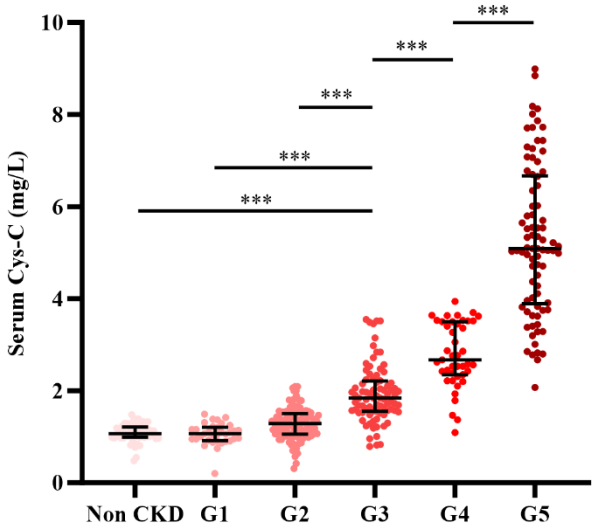
#: The ordinal multiple logistic regression showing variables independently associated with eGFR levels in all participants.

*: The multivariate logistic regression analysis identified the independent influencing factors for high and very-high risk patients with CKD in accordance with KDIGO risk stratification.

The two logistic regression analyses have been adjusted the sex, diabetes, hypertension, and FBG.

FBG: fast blood glucose, BUN: blood urea nitrogen, UA: serum uric acid, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα, eGFR: estimated glomerular filtration rate, CKD: chronic kidney disease; KDIGO: kidney disease improving global outcomes.

a: years, b: mmol/L, c: μmol /L, d: ng/mL, e: mg/L, f: pg/mL.



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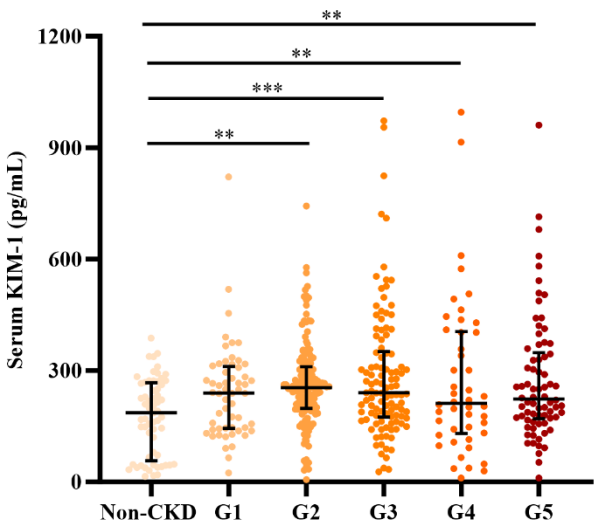

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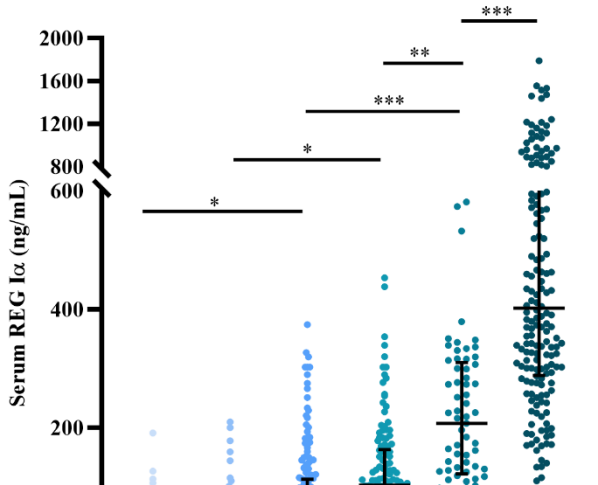

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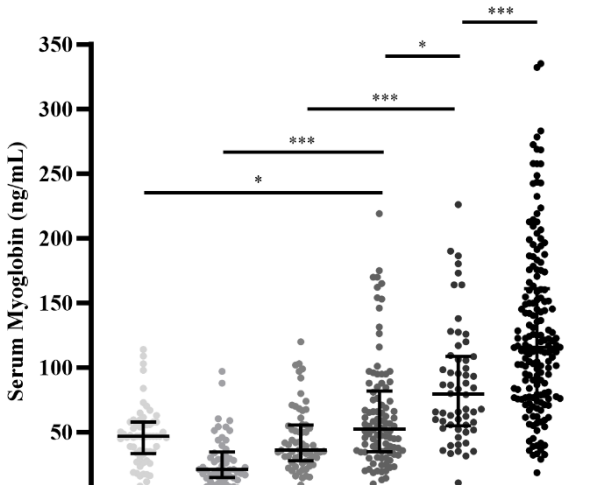

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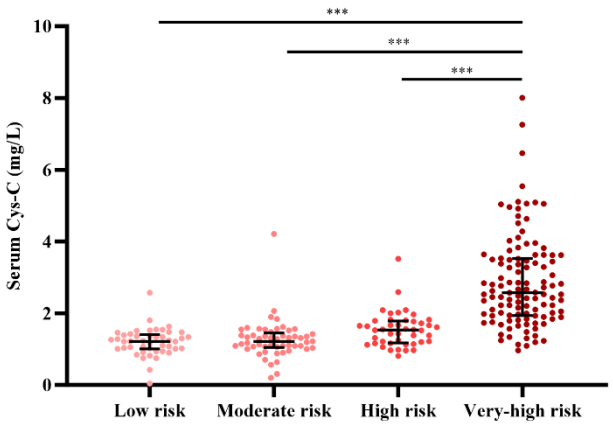

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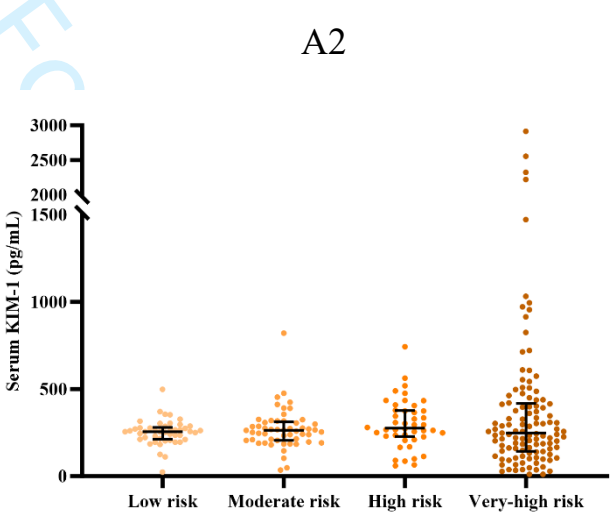

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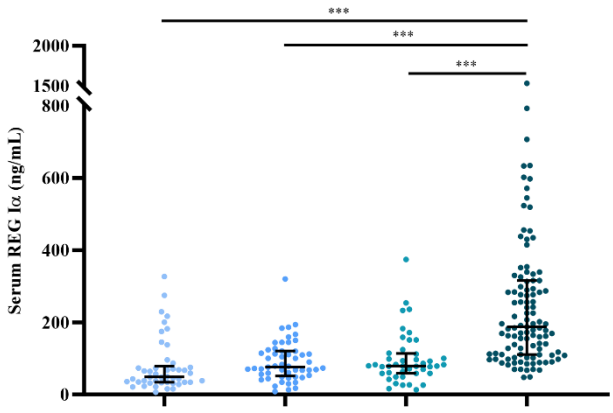

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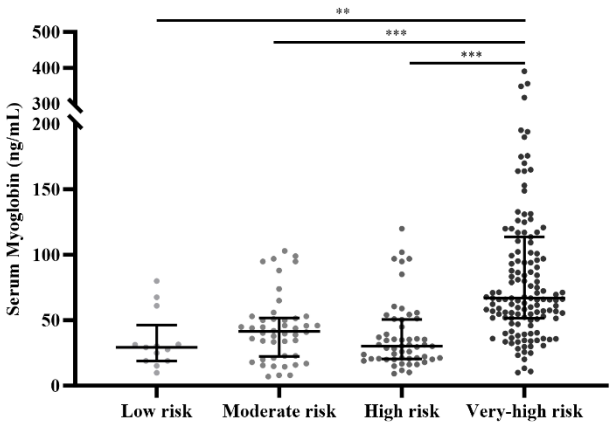

D2

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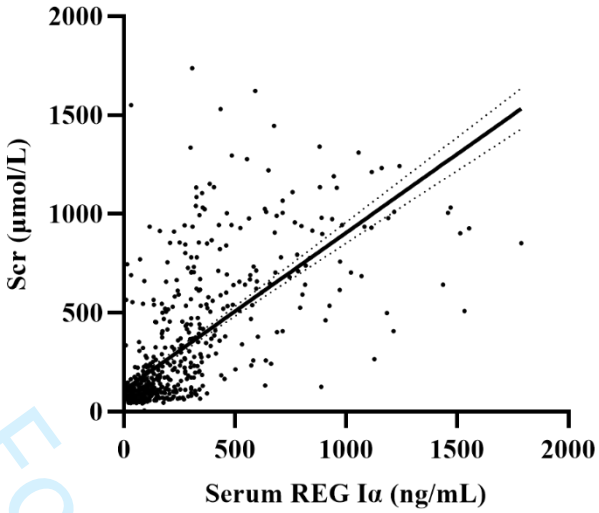

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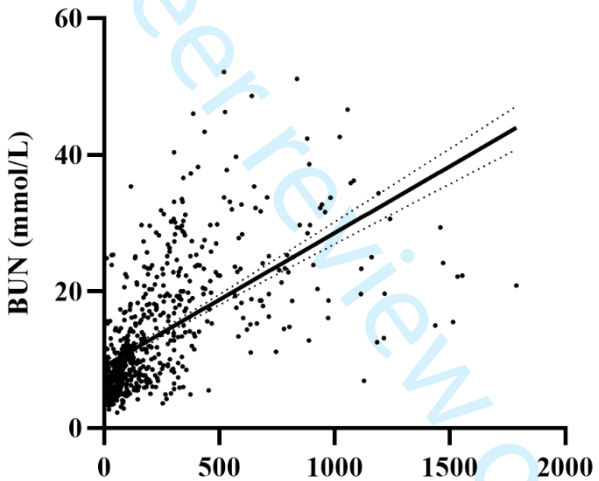

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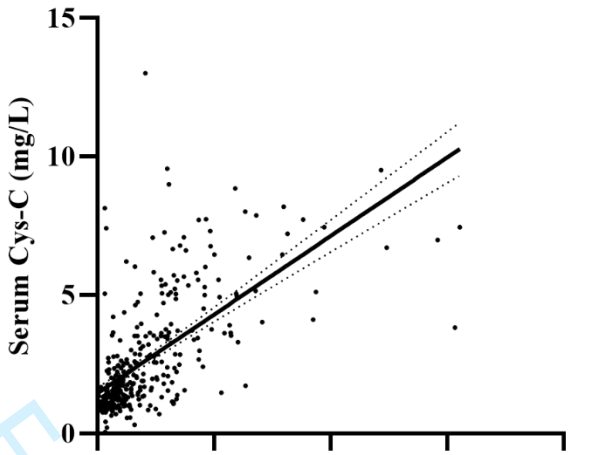

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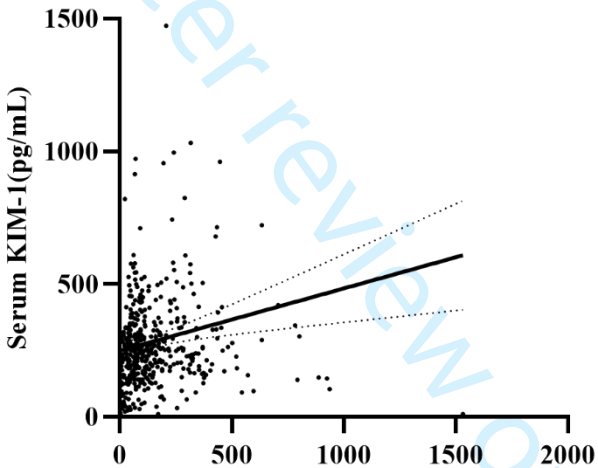

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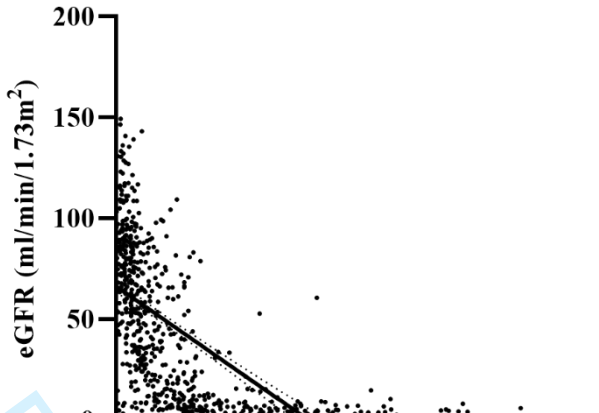
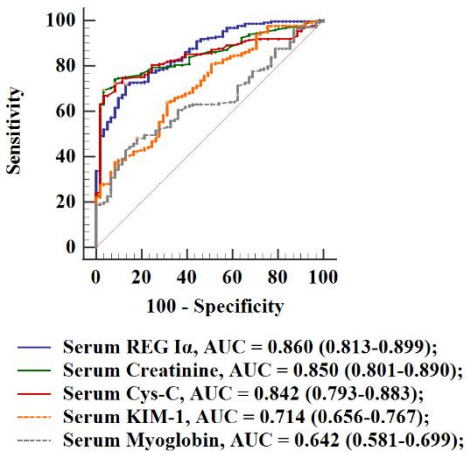


E

Figure 2. Correlations between serum REG Iα and different markers in all participants. A: correlation between serum REG Iα and Scr (r = 0.753, $P < 0.001$), B: correlation between serum REG Iα and BUN (r = 0.733, $P < 0.001$), C: correlation between serum REG Iα and serum Cys-C (r = 0.678, $P < 0.001$), D: correlation between serum REG Iα and serum KIM-1 (r = 0.217, $P < 0.001$), E: correlation between serum REG Iα and eGFR (r = -0.789, $P < 0.001$). Scr: serum creatinine, BUN: blood urea nitrogen, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, eGFR: estimated glomerular filtration rate, REG Iα: regenerating protein Iα.



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Supplementary material

Supplementary data Table 1. Correlations between serum REG Iα and different markers in all participants

Spearman Analyses (r)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age (1)	1.000													
Sex (2)	-0.060	1.000												
Diabetes (3)	0.367***	0.080**	1.000											
Hypertension (4)	0.262***	0.087**	0.236***	1.000										
CVD (5)	0.302***	0.024	0.125***	0.234***	1.000									
FBG (6)	0.258***	0.003	0.567***	0.050	0.062	1.000								
Scr (7)	0.037	0.294***	0.030	0.409***	0.098***	-0.159***	1.000							
BUN (8)	0.157***	0.170***	0.136***	0.442***	0.125***	-0.072**	0.845***	1.000						
UA (9)	-0.086**	0.267***	0.008	0.137***	0.036	-0.051	0.420***	0.379***	1.000					
eGFR (10)	-0.245***	-0.085**	-0.081**	-0.464***	-0.148***	0.123***	-0.927***	-0.856***	-0.328***	1.000				
Serum Cys-C (11)	-0.073	0.048	-0.237***	0.034***	0.079	-0.251***	0.811***	0.741***	0.336***	-0.826***	1.000			
Serum KIM-1 (12)	0.069	0.034	0.141***	0.078	0.031	0.105**	0.091**	0.121***	-0.076	-0.111**	0.111**	1.000		
Serum Myoglobin (13)	0.138***	0.117***	0.098**	0.229***	0.153***	0.024	0.648***	0.606***	0.210***	-0.661***	0.492***	0.066	1.000	

Serum REG Iα (14)	0.154***	0.059	0.073**	0.369***	0.099***	-0.134***	0.753***	0.733***	0.275***	-0.785***	0.678***	0.217***	0.565***	1.000

CVD: chronic kidney disease, FBG: fast blood glucose, Scr: serum creatinine, BUN: blood urea nitrogen, UA: serum uric acid, eGFR: estimated glomerular filtration rate, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα. **: $P < 0.050$, ***: $P < 0.001$.

Supplementary data Table 2. Distribution of serum Cys-C, serum KIM-1, serum REG Ⅰα and serum Myoglobin in KDIGO risk stratification.

KDIGO Risk Stratification Groups	**Low Risk (18.00%)**	**Moderate Risk (20.70%)**	**High Risk (17.20%)**	**Very-high Risk (44.10%)**
Serum Cys-C (mg/L)	1.23 (1.01-1.43)	1.22 (1.05-1.45)	1.54 (1.17-1.79)	2.57 (1.94-3.53)
Serum KIM-1 (pg/mL)	255.77 (211.33-279.86)	262.46 (205.21-312.24)	275.72 (227.08-377.36)	247.28 (142.72-417.99)
Serum Myoglobin (ng/mL)	29.30 (18.97-46.25)	41.50 (22.39-51.82)	30.25 (20.60-50.52)	67.00 (51.70-113.68)
Serum REG Ⅰα (ng/mL)	48.86 (34.18-78.28)	76.05 (51.09-120.54)	79.18 (58.64-113.76)	184.38 (108.81-314.71)

Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ⅰα: regenerating protein Ⅰα, KDIGO: kidney disease improving global outcomes.

The data were presented in quartiles.

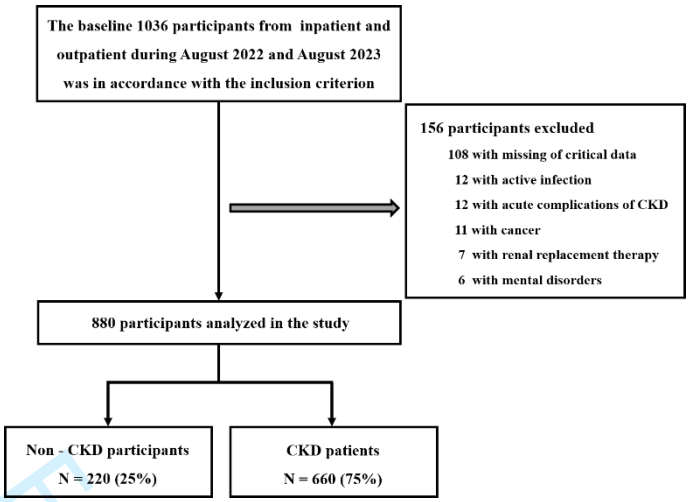
Supplementary data Table 3. Ability of different biomarkers to screen patients with CKD.

Variables	AUC (95% CI)	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Ability of screening patients with CKD in all participants						
Serum REG Iα (ng/mL)	0.860 (0.813-0.899)	70.82	71.63	86.89	41.30	46.85
Serum Creatinine (μmol/L)	0.850 (0.801-0.890)	88.00	69.23	96.72	72.33	47.90
Serum Cys-C (mg/L)	0.842 (0.793-0.883)	1.33	72.12	91.80	55.57	49.09
Serum KIM-1 (pg/mL)	0.714 (0.656-0.767) ***	232.98	63.94	68.85	28.93	35.09
Serum Myoglobin (ng/mL)	0.642 (0.581-0.699) ***	59.70	48.08	81.97	33.50	30.72
Ability of screening high and very-high risk patients according to KDIGO risk stratification in CKD participants						
Serum REG Iα (ng/mL)	0.769 (0.712-0.819)	76.05	82.80	62.63	47.38	69.32
Serum Cys-C (mg/L)	0.865 (0.817-0.904) ***	1.63	75.16	92.93	80.65	70.00
Serum KIM-1 (pg/mL)	0.528 (0.465-0.590) ***	327.10	36.94	87.88	51.69	46.49

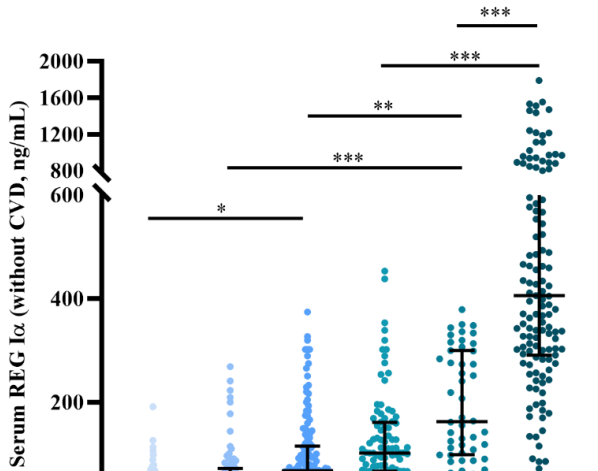
CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ⅰα: regenerating protein Ⅰα.

AUC: area under the receiver operating characteristic curve, PPV: positive predictive value, NPV: negative predictive value.

Comparing with serum REG Iα. ***: $P < 0.001$.



Supplementary data Figure 1. Flowchart of participant selection. In the 1036 inpatient and outpatient participants, 880 were eligible for inclusion. Based on the exclusion criterion, participants were excluded because of missing critical data (n=108), active infection (n=12), acute complications of CKD (n=12), cancer (n=11), renal replacement therapy (n=7), and mental disorders (n=6). CKD: chronic kidney disease.



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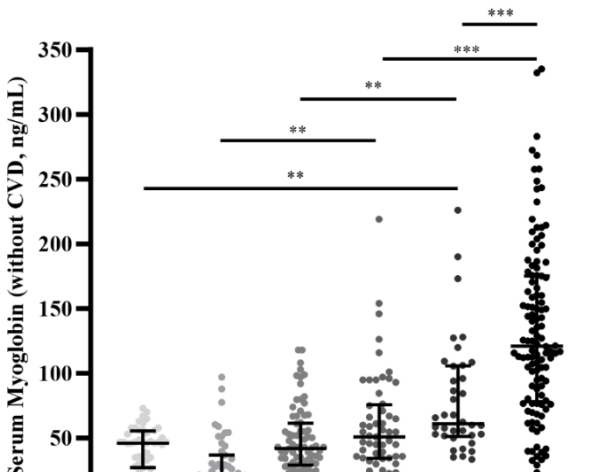
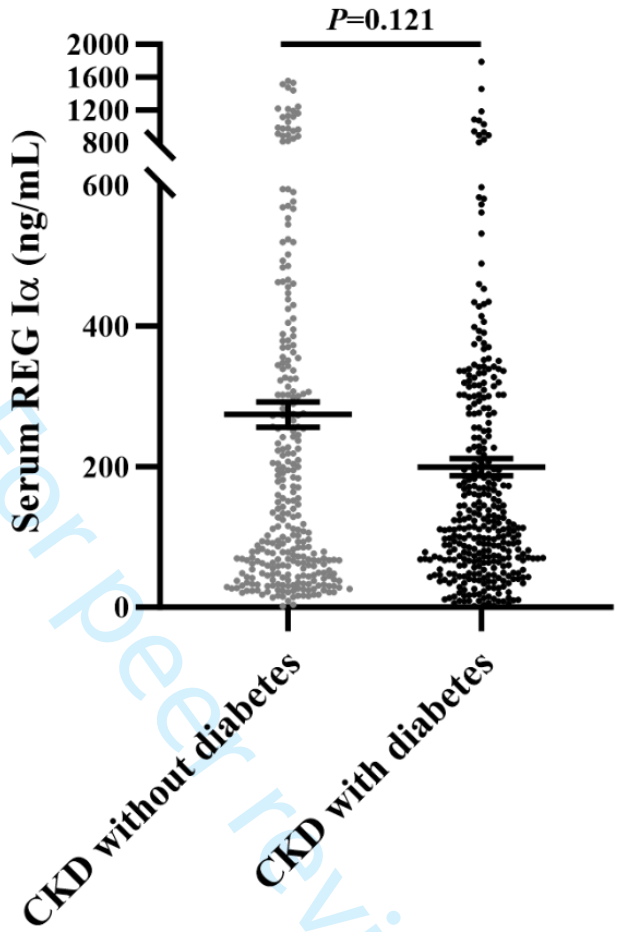


B

Supplementary data Figure 2. Distribution of serum REG Iα and Myoglobin in non-CKD and different CKD groups, which participants without CVD. A: Distribution of serum REG Iα. The serum REG Iα level of non-CKD group was significantly lower than that of G2, G3, G4, and G5 groups individually. B: Distribution of serum myoglobin. The serum myoglobin level of non-CKD was significantly lower than that of G4 and G5 groups individually. CKD: chronic kidney disease, CVD: cardiovascular disease, REG Iα: regenerating protein Iα. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$.



Supplementary data Figure 3. Levels of serum REG Iα in CKD patients with and without T2DM. The median with interquartile range of serum REG Iα was 144.37 (54.71-357.59) ng/ml in CKD patients without T2DM, and 116.57 (65.86-276.34) ng/ml. REG Iα: regenerating protein Iα, T2DM: type 2 diabetes mellitus.

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

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Complete List of Authors:	Huang, Nan; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute Zhu, Xiang; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute Shu, Zhiyi; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute Chen, Sheng; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute Wu, Xiaodong; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute Wang, Hui; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute Huang, Xi; Southeast University Zhongda Hospital, Department of Endocrinology, Zhongda Hospital, School of Medicine Hu, Xiuxiu; Southeast University, Department of Pathology, School of Medicine Sun, Jinfang; Southeast University, Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health Chen, Pingsheng; Southeast University, Department of Pathology, School of Medicine Graf, Rolf; University Hospital Zurich, Department of Surgery Bai, Jianling; Nanjing Medical University, Department of Biostatistics, School of Public Heath Wang, Bin; Southeast University Zhongda Hospital, Department of Nephrology Li, Ling; Southeast University, Department of Endocrinology, Zhongda Hospital, School of Medicine; Southeast University, Pancreatic Research Institute
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Association between elevated serum REG Iα levels and eGFR decline in patients with chronic kidney disease: a cross-sectional study in eastern China

Author

Nan Huang[1, 2#], Xiangyun Zhu[1, 2#], Zhiyi Shu[1, 2#], Sheng Chen[1, 2], Xiaodong Wu[1, 2], Hui Wang[1, 2], Xi Huang[1], Xiuxiu Hu[3], Jinfang Sun[4], Pingsheng Chen[3], Rolf Graf[5], Jianling Bai[6*], Bin Wang[7*], Ling Li[1, 2*]

Address

1. Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China.

2. Pancreatic Research Institute, Southeast University, Nanjing, Jiangsu 210009, China.

3. Department of Pathology, School of Medicine, Southeast University, Nanjing 210009, China.

4. Key Laboratory of Environmental Medicine Engineering, Ministry of Education, School of Public Health, Southeast University, Nanjing, Jiangsu 210009, China.

5. Department of Visceral and Transplantation Surgery, University Hospital of Zurich, Zurich, 8091, Switzerland.

6. Department of Biostatistics, School of Public Heath, Nanjing Medical University, Nanjing, Jiangsu, 211166, China.

7. Department of Nephrology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China.

Correspondence

# These authors have contributed equally to this article.

* Address correspondence to:

1. Ling Li*: M.D.
Department of Endocrinology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China. (e-mail: lingli@seu.edu.cn);

2. Bin Wang*: Ph.D.
Department of Nephrology, Zhongda Hospital, School of Medicine, Southeast University, Nanjing, Jiangsu, 210009, China. (email: wangbinhewei@126.com);

3. Jianling Bai*: Ph.D.

Department of Biostatistics, School of Public Heath, Nanjing Medical University, Nanjing, Jiangsu, 211166, China. (email: baijianling@njmu.edu.cn);

Abstract

Objectives

This study aimed to investigate the relationship between serum regenerating protein Iα (REG Iα) levels and estimated glomerular filtration rate (eGFR) and to evaluate the diagnostic efficiency of REG Iα in chronic kidney disease (CKD).

Design

A cross-sectional study.

Setting

Conducted in eastern China between August 2022 and August 2023.

Participants

A total of 880 participants aged over 18 years were enrolled, with 220 non-CKD participants (111 in male, 50.45%) and 660 CKD patients (366 in male, 55.45%). CKD was diagnosed based on the Kidney Disease: Improving Global Outcomes (KDIGO) 2012 guidelines. Exclusion criteria included participation in other trials, acute kidney injury, end-stage kidney disease undergoing renal replacement therapy, pregnancy, active infections, gastrointestinal or pancreatic inflammation, history of gastrointestinal or pancreatic resections, cancer, and mental disorders.

Results

Serum REG Iα was significantly higher in CKD group (125.54 [60.28-303.39] ng/mL) compared to those in non-CKD group (24.62 [14.09-37.32] ng/mL, $P < 0.001$). Positive correlations were observed between serum REG Iα and serum creatinine (Scr), cystatin C (Cys-C), and kidney injury molecular-1(KIM-1), while a negative correlation was identified with eGFR. After adjusting for sex, diabetes, hypertension, and fasting blood glucose (FBG), the multivariate regression analysis demonstrated a significant association between serum REG Iα and eGFR (OR=1.737 [1.263-2.388], $P = 0.001$). Furthermore, serum REG Iα levels increased progressively with declining kidney function categorized by eGFR ($P < 0.001$). In CKD screening, serum REG Iα demonstrated strong diagnostic performance, with an area under the receiver operating characteristic curves (AUC) of 0.860 (0.813-0.899), providing a sensitivity of 71.63%,

a specificity of 86.89%, a positive predictive value of (PPV) 94.30%, and a negative predictive value (NPV) of 46.85%. Additionally, serum REG Iα exhibited an AUC of 0.769 (0.712-0.819) for identifying high and very-high risk CKD based on KDIGO risk stratification. Its sensitivity significantly outperfomed serum Cys-C and KIM-1 (82.80% vs 75.16% and 36.94%,respectively).

Conclusions

This study provided compelling evidence that serum REG Iα levels were notably elevated in patients with CKD and were closely associated with kidney function. REG Iα may serve as as a promising biomarker for CKD detection and risk stratification.

Clinical trial registration

The study was approved by Ethics Committee of Zhongda Hospital (Approval Number: 2022ZDSYLL204-P01) and conducted in compliance with the Helsinki Declaration. The clinical trial was registered under ChiCTR2300072247.

Data availability statement

The data underlying this article is available from the corresponding author under reasonable request.

Patient and public involvement statement

It was not appropriate or possible to involve patients or the public in the design, or conduct, or reporting, or dissemination plans of our research.

Strengths and limitations of this study

- This study utilized robust logistic regression models to adjust for confounding factors and analyze the relationship between serum REG Iα and kidney function.
- It was the first time to apply KDIGO risk stratification to explore the potential association between serum REG Iα and the risk of CKD progression.
- The DeLong test was applied to statistically compare AUC values among the biomarkers of CKD, enchancing the reliability of diagnostic performance assessments.

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Competing interests' statement

The authors declare that they have no competing interests.

Keywords

Regenerating protein Iα, Chronic kidney disease, Biomarker, Kidney function, Risk stratification;

Abbreviations

List of abbreviations	
Area under the ROC	AUC
Blood urea nitrogen	BUN
Chronic kidney disease	CKD
Chronic kidney disease epidemiology collaboration	CKD-EPI
Cardiovascular disease	CVD
Cystatin C	Cys-C
Diabetic kidney disease	DKD
Estimate glomerular filtration rate	eGFR
End-stage kidney disease	ESKD
Fast blood glucose	FBG
Kidney disease improving global outcomes	KDIGO
Kidney injury molecular-1	KIM-1
Pancreatic stone protein	PSP
Regenerating protein Iα	REG Iα
Receiver operating characteristic curves	ROC
Serum creatinine	Scr
Standard deviation	SD
Uric acid	UA
Urine albuminuria creatine ratio	UACR
Unilateral ureteral obstruction	UUO

Introduction

Chronic kidney disease (CKD) encompasses a wide range of underlying etiologies and exhibits variable progression rates, [1,2] and may become the fifth leading cause of death worldwide by 2040.[3] The endpoint of CKD, known as end-stage kidney disease (ESKD), is characterized by a loss of approximately 90% of kidney function, rendering long-term survival without renal replacement therapy immpossible.[2] The high prevalence, low detection rate, severe clinical outcomes, and substantial econimic burden of CKD underscore its importance as a critical global health issue.[4] Early prevention, detection, and treatment are key to improving patient outcomes and slowing the progression to ESKD.

Current biomarkers such as serum creatinine (Scr), blood urea nitrogen (BUN), estimate glomerular filtration rate (eGFR), and urine albuminuria creatine ratio (UACR) are routinely used to evaluate CKD severity.[5-8] In 2012, the Kidney Disease: Improving Global Outcomes (KDIGO) has taken the eGFR and UACR to create risk stratification strategies, assisting in the tailored management of CKD and guiding treatment for those at a higher risk of progression.[9] Emerging biomarkers, including cystatin C (Cys-C), kidney injury molecular-1 (KIM-1), and β2-microglobuline, have demonstrated potential ability in enhancing the precision of CKD screening, either independently or in conjunction with traditional markers.[2,3,10,11] However, most biomarkers have not yet met clinical expectations in terms of sensitivity, specificity, and practicality.[12-15] Few biomarkers are capable of effectively detecting CKD while simultaneously assessing progression risk. Therefore, identifying a novel biomarker can both monitor kidney function decline and stratify CKD progression risk remains of paramount importance.

Regenerating protein Iα (REG Iα), a 16 kDa protein primarily secreted by the pancreas and intestine,[16] is also referred to as pancreatic stone protein (PSP).[17] It plays a vital role in cellular proliferation and regeneration processes.[18,19] Recent studies have reported the presence of REG Iα in patients with various kidney diseases, suggesting its involvement in renal pathology.[20,21] Our previous studies also have further demonstrated that serum REG Iα levels are elevated in patients with diabetic kidney

disease (DKD), consisdent with findings of H. Sobajima.[22-24] These observations highlight the potential role of REG Iα as a biomarker for kidney insufficiency.

In this study, we aimed to investigate the relationship between serum REG Iα levels and kidney function, assessed its potential as a screening tool for CKD, and evaluate its role as a biomarker for kidney function and disease progression.

Methods

Study subjects

Participants were recruited from Zhongda Hospital between August 2022 and August 2023. The study was approved by the ethics committee (Approval Number: 2022ZDSYLL204-P01), with a clinical study registration of ChiCTR2300072247. Informed consent was acquired from all participants.

The inclusion criteria were as follows: (1) non-CKD participant: age > 18 years; (2) CKD patients: age > 18 years and diagnosed with CKD in accordance to the Kidney Disease: Improving Global Outcomes (KIDGO) 2012 guidelines.[1] The exclusion criteria were as follows: (1) enrolled in another trial; (2) acute kidney injury; (3) patients with ESKD who are undergoing renal replacement therapy; (4) pregnancy; (5) active infection; (6) acute or chronic inflammation of the gastrointestinal system and pancreas; (7) history of gastrointestinal or pancreatic resections; (8) cancer; (9) mental disorders. eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[25] CKD stages were classified based on the U.S. National Kidney Foundation, while risk stratification of CKD progression was performed according to the KDIGO guideline.[26,27] Subgroup analyses were conducted among CKD patients to explore the relationship between serum REG Iα and different degrees of kidney function impairment.

A sample size calculation was conducted to ensure sufficient statistical power to detect associations between serum REG Iα and eGFR. The parameters included an expected elevated REG Iα proportion (P1=0.3), a two-tailed significance level (α=0.05), statistical power (80%), and finite population correction. A total of 880 participants were included to enhance the robustness and generalizability of the study.

Data collection and quality assessment

Baseline demographics data were collected using standardized questionnaires. All participants have undergone 12-hours fasting and taken about 3mL of peripheral blood sample. Blood samples were centrifuged at 35oo rpm for 15 min, and the upper serum was collected within 6-hours. Serum samples were immediately stored at -80℃ for subsequent analyses. Clinical biochemical parameters were extracted from the clinical laboratory of Hospital, such as Scr, BUN, uric acid (UA), Cys-C, Myoglobin, UACR, and fast blood glucose (FBG). The laboratory implements internal and external quality control procedures directed by a Chinese Quality Control Laboratory. The serum REG Iα levels were determined using a double antibody sandwich ELISA, as previously described.[28] Serum KIM-1 was measured using an ELISA kit (KE00136) from Proteintech.

Statistical analysis

Statistical analyses were conducted using SPSS 20.0, Med-Calc, and GraphPad Prism 8.0. Continuous variables were summarized as mean ± standard deviation (SD) for normally distributed data or as median with interquartile range (IQR) for non-normally distributed data. For categorical variables, the frequency with a percentage of each category was calculated. Normality was assessed using graphical methods (Q-Q plots) and the Shapiro-Wilk test. For variables that did not meet normality, we used non-parametric descriptors and methods. We used Student's t test for normally distributed continuous variables, Mann Whitney U test for non-normally distributed continuous variables, and chi-square or Fisher's exact test for categorical variables for two group comparisons. Tukey's multiple comparison test was employed to examine the differences in biomarker values across the three or more groups, thereby avoiding the issue of multiple comparisons. 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. 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. 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 study assessed evaluate accuracy using sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV), and accuracy, and applied the DeLong test to statistically analyze AUC differences between receiver operating characteristic curves. *P* value of <0.050 using two-tailed tests was considered statistically significant.

Results

Baseline characteristics of the study population

Overall, a total of 880 participants were enrolled, comprising 220 non-CKD participants and 660 CKD patients (Supplementary data Figure 1). Significant differences were observed between CKD patients and non-CKD participants in terms of age, complication diseases (diabetes and hypertension), and kidney function biomarkers (Scr, BUN, UA, eGFR, Cys-C, and KIM-1), and serum myoglobin (all $P < 0.001$, Table 1). Serum REG Iα levels were significantly elevated in CKD patients (125.54 [60.28, 303.39] ng/mL) compared to non-CKD participants (24.62[14.09, 37.32] ng/mL, $P < 0.001$, Table 1). Biomarker trends revealed that serum Cys-C, REG Iα, and myoglobin levels progressively increased as eGFR declined (Figure 1: A1, C1, and D1). Serum KIM-1 levels also exhibited significant differences between non-CKD group and CKD groups (G2 to G5, $P < 0.010$, Figure 1: B1). Further analyses confirmed that cardiovascular disease (CVD, Supplementary data Figure 2) did not influence the distribution of serum REG Iα and myoglobin levels. Diabetes had no influence on serum REG Iα in CKD patients (Supplementary data Figure 3) .

Table 1. Clinical characteristics of study population at baseline examination.

	Non-CKD Participants	CKD Patients	*P* Value
Number	220	660	-
DEMOGRAPHICS			
Age (years)	53 (40-62)	62 (50-72)	< 0.001
Sex (male, %)	111 (50.45)	366 (55.45)	0.212
Smoking (yes, %)	41 (18.64)	142 (21.52)	0.141
Drinking (yes, %)	30 (13.64)	84 (12.73)	0.823
COMPLICATION DISEASES			
Diabetes (yes, %)	75 (34.09)	350 (53.03)	< 0.001
Hypertension (yes, %)	65 (29.55)	501 (75.91)	< 0.001
Cardiovascular disease (CVD, yes, %)	44 (20.00)	149 (22.58)	0.453
LABORATORY MEASUREMENTS			
Fast blood glucose (FBG, mmol/L)	5.45 (4.99-6.67)	5.34 (4.57-7.11)	0.008
Serum creatinine (Scr, μmol/L)	64.00 (55.25-76.00)	126.00 (83.00-418.50)	< 0.001
Blood urea nitrogen (BUN, mmol/L)	5.20 (4.30-6.28)	10.30 (7.00-18.60)	< 0.001
Serum uric acid (UA, μmol/L)	301.00 (256.00-365.25)	354.00 (290.00-443.00)	< 0.001
Estimated glomerular filtration rate (eGFR, mL/min per 1.73 m^2)	102.37 (95.73-112.36)	44.03 (10.96-77.09)	< 0.001
Serum cystatin C (Cys-C, mg/L)	1.10 (0.99-1.24)	1.79 (1.23-3.50)	< 0.001
Serum kidney injury molecular-1 (KIM-1, pg/mL)	186.28 (57.22-266.88)	247.72 (175.10-334.13)	< 0.001
Serum Myoglobin (ng/mL)	46.81 (33.49-58.00)	64.04 (35.96-112.51)	< 0.001
Serum regenerating protein Iα (REG Iα, ng/mL)	24.62 (14.09-37.32)	125.54 (60.28-303.39)	< 0.001

CKD: chronic kidney disease; The data were presented in quartiles. Student's t tests were used for normally distributed continuous variables. Mann Whitney U tests were used for non-normally distributed continuous variables. And chi-square or Fisher's exact tests were used for categorical variables for two group comparisons.

Relationship between serum REG Iα and kidney function

This study explored the relationship between serum REG Iα levels and kidney function biomarkers. In figure 2, Spearman correlation analyses demonstrated a strong positive association between serum REG Iα and Scr (r = 0.753, $P < 0.001$), BUN (r = 0.733, $P < 0.001$), serum Cys-C (r = 0.678, $P < 0.001$), and serum KIM-1 (r = 0.217, $P < 0.001$). And a siginificant negative correlation was observed between serum REG Iα and eGFR (r = -0.789, $P < 0.001$). A comprehensive summary of these correlations was provided in supplementary data Table 1. Ordinal logistic regression analysis carried out in all participants, with eGFR as a grade-dependent variable, revealed that serum REG Iα/100 levels was significantly associated with eGFR (OR=1.737, 95% CI: 1.263-2.388, $P = 0.001$, Table 2).

Table 2. The logistic regression analyses showing the relationship between variables and kidney function.

	Ordinal Logistic Regression#		Multivariate Logistic Regression*	
	***P* Value**	**OR (95% CI)**	***P* Value**	**OR (95% CI)**
Age[a]	0.050	1.020 (1.001-1.041)	0.009	0.966 (0.942-0.992)
BUN[b]	< 0.001	1.266 (1.165-1.376)	< 0.001	1.440 (1.216-1.706)
UA[c]	0.337	1.001 (0.999-1.004)	0.085	1.003 (1.000-1.007)
Serum Myoglobin[d]	0.165	1.005 (0.998-1.013)	0.148	1.136 (0.897-1.559)
Serum Cys-C[e]	< 0.001	6.784 (4.016-11.460)	0.071	1.853 (0.949-3.620)
Serum KIM-1/100[f]	0.133	1.069 (0.980-1.167)	0.122	1.243 (0.943-1.639)
Serum REG Iα/100[d]	0.001	1.737 (1.263-2.388)	0.022	1.799 (1.088-2.975)

#: The ordinal multiple logistic regression showing variables independently associated with eGFR levels in all participants. *: The multivariate logistic regression analysis identified the independent influencing factors for high and very-high risk patients with CKD in accordance with KDIGO risk stratification. The analyses 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.

FBG: fast blood glucose, BUN: blood urea nitrogen, UA: serum uric acid, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα, eGFR: estimated glomerular filtration rate, CKD: chronic kidney disease; KDIGO: kidney disease improving global outcomes.

a: years, b: mmol/L, c: μmol /L, d: ng/mL, e: mg/L, f: pg/mL.

Subgroup analysis in patients with CKD

To explore the relationship between serum REG Iα levels and CKD progression risk, 256 CKD patients were included in the sub-research and classified by eGFR and UACR levels in accordance with KDIGO risk stratification guideline.[1] The patients were stratified into four KDIGO risk groups: low risk (18.00%), moderate risk (20.70%), high risk (17.20%), and very-high risk (44.10%, supplementary data Table 2). Serum REG Iα levels significantly increased with higher CKD risk categories, reaching 184.38 (108.81, 314.71) ng/mL in the very-high risk group ($P < 0.001$, Figure 1: C2). Similar trends were observed for serum Cys-C (2.57 [1.94, 3.53] mg/L) and myoglobin (67.00 [51.70, 113.68]ng/mL, $P < 0.001$, Figure 1: A2, D2). However, the serum KIM-1 did not exhibit an increasing trend ($P > 0.050$, Figure 1: B2). Multiple logistic regression analysis demonstrated that serum REG Iα/100 was an independent influencing factor for high and very-high CKD risk (OR=1.799, 95% CI: 1.088-2.975, $P = 0.022$, Table 2) .

Ability of serum REG Iα in screening patients with kidney dysfunction

Receiver operating characteristic (ROC) analysis evaluated the utility of serum REG Iα as a screening tool for CKD and its ability to stratify CKD risk (Figure 3 and supplementary data Table 3). Serum REG Iα demonstrated an AUC of 0.860 (95% CI: 0.813-0.899) for detecting CKD, comparable to serum creatinine (0.850, 95% CI: 0.801-0.890) and serum Cys-C (0.842, 95% CI: 0.793-0.883). At a cutoff value of 70.82 ng/mL, serum REG Iα had a sensitivity of 71.63%, specificity of 86.89%, positive predictive value (PPV) of 94.30%, and negative predictive value (NPV) of 46.85%. Serum KIM-1 showed a lower AUC than serum REG Iα, measuring 0.714 (95% CI: 0.656-0.767, $P < 0.001$). Serum myoglobin had the lowest AUC among the five biomarkers, measuring 0.642 (95% CI: 0.581-0.699, $P < 0.001$).

For distinguishing high and very-high CKD risk, serum REG Iα demonstrated superior performan compared to serum KIM-1 (AUC = 0.769 [0.712-0.819] vs 0.528 [0.465-0.590], $P < 0.010$, supplementary data Table 3). Serum Cys-C had the highest AUC (0.865 [0.817-0.904], $P < 0.010$) among the three biomarkers. Serum REG Iα,

with a cutoff value of 76.05 ng/mL, exhibited sensitivity of 82.80%, specificity of 62.63%, PPV of 77.38%, and NPV of 69.32%. Notably, serum REG Iα showed significantly higher sensitivity than serum Cys-C and KIM-1.

Discussion

This study was the first time to systematically evaluate serum REG Iα levels in a broad CKD population and confirm its upregulation in CKD patients. First, serum REG Iα levels increased progressively with declining eGFR and correlated strongly with conventional kidney function biomarkers (Scr, BUN, Cys-C, and KIM-1). Second,serum REG Iα emerged as an independent risk factor for patients classified as high and very-high risk according to the KDIGO risk stratification. Third, serum REG Iα demonstrated robust diagnostic performance, showing higher sensitivity than serum KIM-1 for identifying CKD and distinguishing risk progression.

REG Iα, a low molecular weight protein (16 kDa), was initially discovered in pancreas, and was identified as pancreatic stone protein (PSP) due to its role in inhibiting calcium carbonate stone formation in pancreatic ducts.[29,30] Immunohistochemical studies have shown overexpression of REG Iα in impaired kidneys, particularly in proximal tubules and thick ascending limbs of Henle's loops.[20] Previous studies also reported elevated REG Iα levels in diabetic kidney disease (DKD), suggesting its involvement in tubular dysfunction and kidney injury.[22,31,32] Our previous studies provided evidence, that serum REG Iα levels were notably elevated in patients with type 2 diabetes mellitus who had kidney dysfunction. And we also observed that pregnant women with early renal dysfunction exhibited similar elevations in serum REG Iα levels.[24,33] Notably, the serum REG Iα levels remained significantly elevated when the scope of this study was expanded to the entire CKD population.

We incorporated myoglobin (17.6 kDa)[34] to investigate whether the elevated level of serum REG Iα represents a universal phenomenon of accumulation in CKD. The myoglobin was identified as a biomarker for acute myocardial ischemia and rhabdomyolysis.[35] Some studies indicated that serum myoglobin levels increase in

kidney impairment. This accumulation was primarily attributed to slower clearance in CKD.[36] We showed that serum levels of myoglobin have no differences between non-CKD participants and patients with early stage of CKD (group G1and group G2). And there was a gradual increase in patients with medium to end-stages of CKD (group G3 to group G5). The possible reason was as follows: in the early stages of kidney injury, compensatory glomerular hypertrophy with hyperfiltration state was known to exist. This mechanism allowed some low molecular weight protein to be filtered through the glomerular basement membrane, resulting in no discernible difference during the early stage of CKD (eGFR $\geq$ 60 mL/min per 1.73 m^2), and even appearing as a transient decrease. With progression to medium to end-stages of CKD (eGFR < 60 mL/min per 1.73 m^2), the glomerular basement membrane thickened and led to significant decline in glomerular filtration function, ultimately causing proteins accumulation in serum.[37] The serum levels of REG Iα showed a gradual increase in medium to end-stages of CKD, which was similar to the rise observed in serum myoglobin levels. However, it exhibited a significant increase in the early stage of CKD, which was different with serum myoglobin. This suggested that, in addition to the sensitive accumulation of serum REG Iα as a low molecular weight protein, there might be a specific production when it comes to kidney impairment. The unique behavior of serum REG Iα, compared to low molecular weight protein, highlights its dual role: accumulation due to reduced glomerular clearance and potential increased production in response to kidney injury. Unlike myoglobin, which remains stable in early CKD stages, serum REG Iα levels were significantly elevated even at the ultra-early stage of kidney dysfunction, suggesting a sensitive and specific response to renal impairment.

The etiology underlying the upregulation of REG Iα production in CKD patients remains elusive. Two potential mechanisms are considered as follows. First, REG Iα resists apoptosis and promotes cell proliferation in different inflammation situations.[38-42] Studies have shown that cytokines such as IL-6 can increase the proliferation of REG Iα, which is involved in cell regeneration and repair.[42,43] In

CKD, the occurrence and progression of the disease are closely related to the expression of multiple inflammatory cytokines, including IL-1, IL-6, and TNF-α.[37,44,45] Under the stimulation of chronic inflammation, different types of renal cells secrete REG Iα locally to participate in kidney anti-apoptosis and proliferation and against kidney fibrosis in the development of CKD. The secreted REG Iα enters the circulation through reabsorption by kidney tubules, resulting in a significant increase in serum REG Iα levels. Thus, REG Iα might serve as an inflammatory factor involved in kidney diseases. Second, REG Iα is primarily synthesized in the pancreas and released into the circulation.[18,46] A hypothesis suggests that a cross-talk mechanism exists between the pancreas and kidney when renal damage occurs. This interaction may prompt the pancreas to produce more REG Iα in response to kidney injury, resulting the elevation in serum. Although the exact cause of REG Iα upregulation remains unclear, these two proposed mechanisms provided potential insights into the relationship between REG Iα and CKD.

At present, the assessment of CKD generally focused on glomerular filtration capacity, which is characterized by Scr, UACR, and eGFR.[2] The stabilities of these factors are compromised by age, dietary intake, and physical activity. In general, the performance of eGFR is poor for populations outside of North America, Australia, and Europe, and accuracy decreases at the extremes in the distribution of age, and body composition.[7] Therefore, new biomarkers of kidney impairment have been discovered. Cys-C is produced by all nucleated cells at a steady rate and freely filtered by the kidney.[47] The accumulation of its levels is observed in case of glomerular filtration dysfunction, with limited impact and strong stability.[48] Eventually, serum Cys-C is incorporated into the eGFR equation as a reliable and sensitive indicator of kidney function. The sensitivity of serum Cys-C is heightened in the early stages of CKD compared to creatinine.[49,50] Another biomarker is KIM-1,[51,52] which is secreted following kidney proximal tubular injury, and has emerged as a sensitive and specific biomarker of acute kidney injury and CKD progression.[53-56] Compared to serum KIM-1, the REG Iα had several advantages as follows. This

paper demonstrated that levels of serum REG Iα increase significantly earlier than KIM-1, making it a better marker for the early detection of renal injury. In addition, serum 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. Serum REG Iα also exhibited better AUC, sensitivity, and specificity, enhancing its diagnostic performance in identifying CKD patients. 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.

In this study, serum REG Iα strongly correlated with serum creatinine and Cys-C, and had a similar performance to serum creatinine and Cys-C in detecting patients with CKD. The serum levels of REG Iα were more likely related to glomerular filtration capacity according to the correlation analyses, although the mechanism of its production and degradation or excretion in CKD remained unclear. We indicated that serum REG Iα might be more sensitive than serum creatinine and Cys-C in detecting ultra-early stage of kidney dysfunction. Similar phenomenon was observed in distribution analyses in KDIGO risk stratification. The KDIGO risk classification was widely used in evaluating the progression and prognosis risk in patients with CKD.[57-60] Serum levels of REG Iα gradually elevated with higher KDIGO risk stratification categories. And it emerged as an independent risk factor for patients with CKD categorized as high and very-high risk. Our findings provided a new insight that serum REG Iα performed better than serum KIM-1 in screening patients with CKD and detecting high and very-high risk patients with CKD. Moreover, serum REG Iα displayed a highest sensitivity in identifying high and very-high risk CKD. These results highlight the potential application of serum REG Iα as a valuable biomarker in the screening of patients with CKD and the assessment of CKD risk.

We observed that patients with CKD were older than non-CKD participants, and they had higher rates of diabetes and hypertension. This finding was consistent with the

typical etiology of CKD. Globally, diabetes and hypertension are recognized as the primary causes of CKD.[2] Diabetes accounts for 30-50% of all CKD cases and affects approximately 285 million adults worldwide. A consistent finding in observational studies shows that the increased risk of developing is associated with blood pressure control. These findings were consistent with the expected proportion of CKD etiology in our study.

There are some limitations in this study. First, the cross-sectional design precluded causal inference. And due to the challenges of obtaining detailed data of renal biopsy, our team have endeavored to conduct a pre-subgroup analysis within the available constraints. We found that CKD etiologies (for example, IgA nephropathy, membranous nephropathy, and diabetic kidney disease) have no effect on serum REG levels. To enhance causality and generalizability, longitudinal designs with larger sample sizes and diverse populations should be considered in forthcoming research. 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, the precise source of elevated REG Iα in CKD remains unclear, warranting further mechanistic investigations. Forth, to address residual confounding, future studies should incorporate additional covariates such as drug use and different causes of CKD and conduct sensitivity analyses to assess potential biases.

Moreover, we acknowledged the potential impact of self-reporting and recall bias in our questionnaire-based demographic data, especially among CKD patients prone to cognitive and emotional challenges. Factors such as cognitive impairment, symptom complexity, emotional stress, health literacy variations, and medication effects can skew reporting accuracy. To mitigate risks, we used standardized questionnaires with clear instructions. However, given CKD's clinical complexity, findings should be interpreted cautiously. Future studies should cross-verify self-reported data with objective clinical measures to enhance accuracy and reliability.

Conclusion

This study provided compelling evidence that serum REG Iα is significantly upregulated in CKD patients and strongly associated with kidney function. Serum REG Iα demonstrated notable diagnostic sensitivity and utility in CKD risk stratification, underscoring its potential as a valuable biomarker for detecting kidney function decline and identifying high risk CKD patients.

Contributorship statement

Professor Ling Li acted as guarantor. Ling Li conceptualized the study; Bin Wang reviewed and edited the manuscript; Nan Huang and Xiangyun Zhu were responsible for the data analysis and wrote the original draft. Nan Huang, Sheng Chen, Xiaodong Wu, Hui Wang, Zhiyi Shu and Xi Huang were responsible for inclusion of population, collection of samples and data, and the experiments. Jianling Bai and Jinfang Sun were responsible for guidance on data analysis. Pingsheng Chen and Xiuxiu Hu were responsible for analysis of kidney biopsy tissues from CKD patients. Rolf Graf provided the excellent technical support for this study.

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Tables

Table 1. Clinical characteristics of study population at baseline examination.

CKD: chronic kidney disease; The data were presented in quartiles. Student's t tests were used for normally distributed continuous variables. Mann Whitney U tests were used for non-normally distributed continuous variables. And chi-square or Fisher's exact tests were used for categorical variables for two group comparisons.

Table 2. The logistic regression analyses showing the relationship between variables and kidney function.

#: The ordinal multiple logistic regression showing variables independently associated with eGFR levels in all participants. *: The multivariate logistic regression analysis identified the independent influencing factors for high and very-high risk patients with CKD in accordance with KDIGO risk stratification. The analyses 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.

FBG: fast blood glucose, BUN: blood urea nitrogen, UA: serum uric acid, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα, eGFR: estimated glomerular filtration rate, CKD: chronic kidney disease; KDIGO: kidney disease improving global outcomes.

a: years, b: mmol/L, c: μmol /L, d: ng/mL, e: mg/L, f: pg/mL.

Figures

Figure 1. Distribution of serum Cys-C, serum KIM-1, serum REG Iα, and serum Myoglobin in different groups.

A1, B1, C1, and D1 are in all participants: the CKD groups were classified in accordance with eGFR levels as described in methods. A1: Distribution of serum Cys-C. B1: Distribution of serum KIM-1. C1: Distribution of serum REG Iα. D1: Distribution of serum myoglobin. A2, B2, C2, and D2 are in patients with CKD: the patients were classified in accordance with 2012 KDIGO risk stratification as described in methods. A2: Distribution of serum Cys-C. B2:

Distribution of serum KIM-1. C2: Distribution of serum REG Iα. D2: Distribution of serum Myoglobin. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$. Tukey's multiple comparison tests were conducted to examine the differences in different groups. CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα.

Figure 2. Correlations between serum REG Iα and different markers in all participants.

A: correlation between serum REG Iα and Scr (r = 0.753, $P < 0.001$), B: correlation between serum REG Iα and BUN (r = 0.733, $P < 0.001$), C: correlation between serum REG Iα and serum Cys-C (r = 0.678, $P < 0.001$), D: correlation between serum REG Iα and serum KIM-1 (r = 0.217, $P < 0.001$), E: correlation between serum REG Iα and eGFR (r = -0.789, $P < 0.001$). Scr: serum creatinine, BUN: blood urea nitrogen, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, eGFR: estimated glomerular filtration rate, REG Iα: regenerating protein Iα.

Figure 3. Ability of screening patients with CKD.

The AUC of serum REG Iα was 0.860 (95% CI: 0.813-0.899), and that of serum Creatinine was 0.850 (95% CI: 0.801-0.890), while that of serum Cys-C was 0.842 (95% CI: 0.793-0.883). Serum KIM-1 had an AUC of 0.714 (95% CI: 0.656-0.767), and serum Myoglobin had an AUC of 0.642 (95% CI: 0.581-0.699). The AUC of serum REG Iα was similar to serum Creatinine and serum Cys-C ($P > 0.050$), and was significantly higher than serum KIM-1 and serum Myoglobin ($P < 0.001$). DeLong tests were applied to analyze AUC differences between receiver operating characteristic curves. CKD: chronic kidney disease, AUC: area under the receiver operating characteristic curve, REG Iα: regenerating protein Iα, Cys-C: cystatin C, KIM-1: kidney injury molecular-1.

Supplementary material

Supplementary data Table 1. Correlations between serum REG Iα and different markers in all participants.

**: $P < 0.050$, ***: $P < 0.001$. CVD: cardiovascular disease, FBG: fast blood glucose, Scr: serum creatinine, BUN: blood urea nitrogen, UA: serum uric acid, eGFR: estimated glomerular filtration rate, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα.

Supplementary data Table 2. Distribution of serum Cys-C, serum KIM-1, serum REG Ⅰα and serum Myoglobin in KDIGO risk stratification.

Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ⅰα: regenerating protein Ⅰα, KDIGO: kidney disease improving global outcomes. The data were presented in quartiles.

Supplementary data Table 3. Ability of different biomarkers to screen patients with CKD.

***: Comparing with serum REG Iα, $P < 0.001$. DeLong tests were applied to analyze AUC differences between receiver operating characteristic curves. CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ⅰα: regenerating protein Ⅰα. AUC: area under the receiver operating characteristic curve, PPV: positive predictive value, NPV: negative predictive value.

Supplementary data Figure 1. Flowchart of participant selection.

In the 1036 inpatient and outpatient participants, 880 were eligible for inclusion. Based on the exclusion criterion, participants were excluded because of missing critical data (n=108), active infection (n=12), acute complications of CKD (n=12), cancer (n=11), renal replacement therapy (n=7), and mental disorders (n=6). CKD: chronic kidney disease.

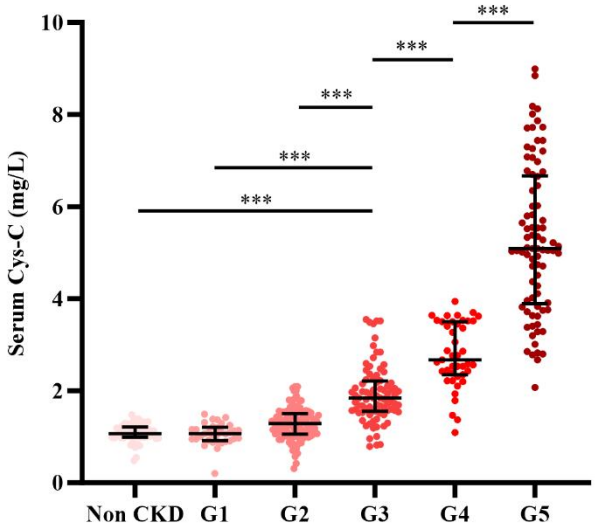
Supplementary data Figure 2. Distribution of serum REG Iα and Myoglobin in non-CKD and different CKD groups, which participants without CVD.

A: Distribution of serum REG Iα. The serum REG Iα level of non-CKD group was significantly lower than that of G2, G3, G4, and G5 groups individually. B: Distribution of serum myoglobin. The serum myoglobin level of non-CKD was significantly lower than that of G4 and G5 groups individually.

CKD: chronic kidney disease, CVD: cardiovascular disease, REG Iα: regenerating protein Iα. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$. Tukey's multiple comparison tests were conducted to examine the differences in different groups.

Supplementary data Figure 3. Levels of serum REG Iα in CKD patients with and without diabetes.

Mann-Whitney U test was used for non-normally distributed continuous variables ($P = 0.121$). REG Iα: regenerating protein Iα.



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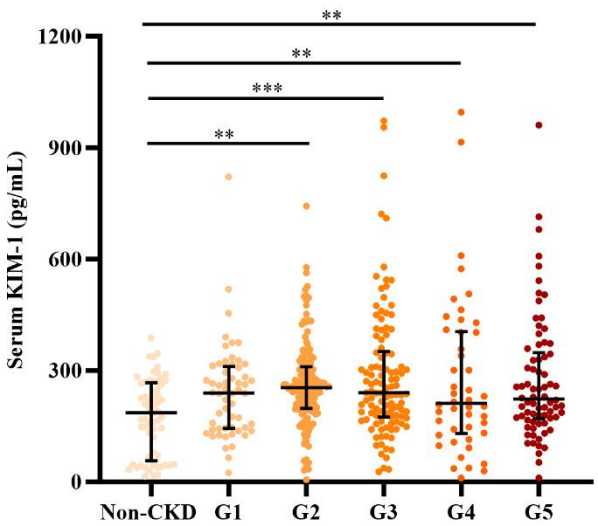

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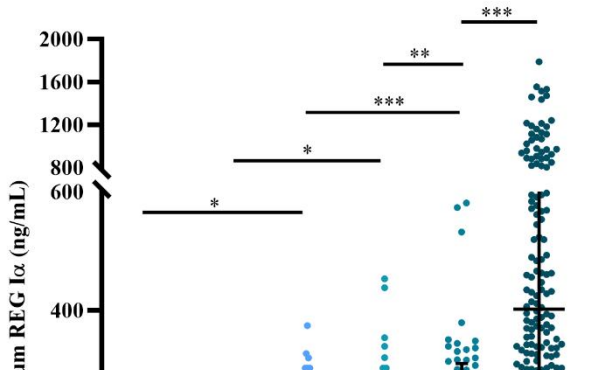

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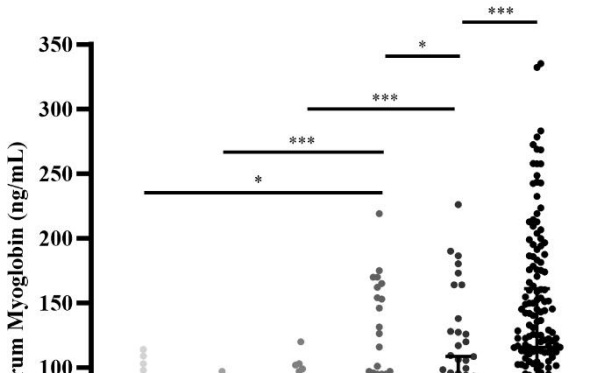

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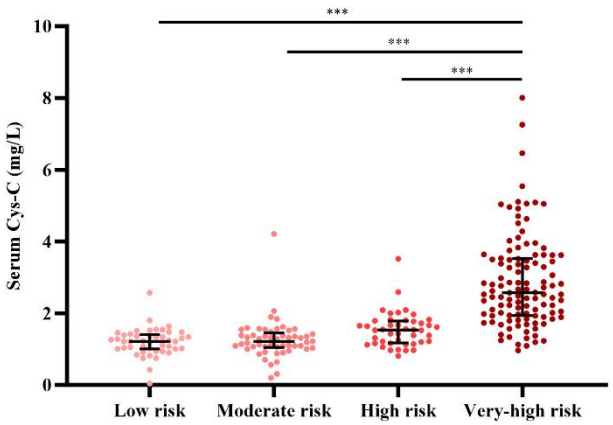

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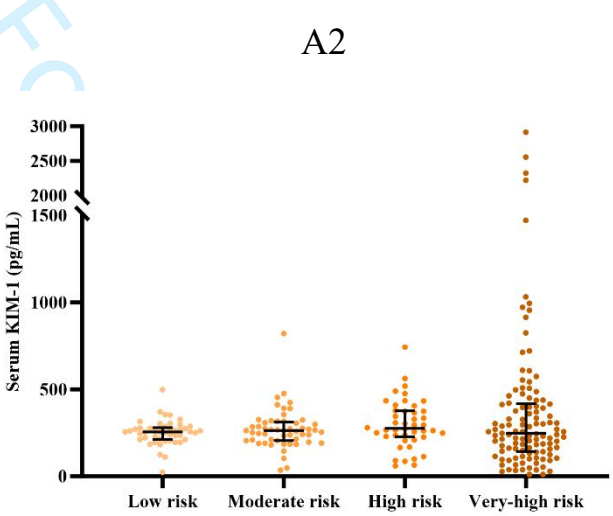

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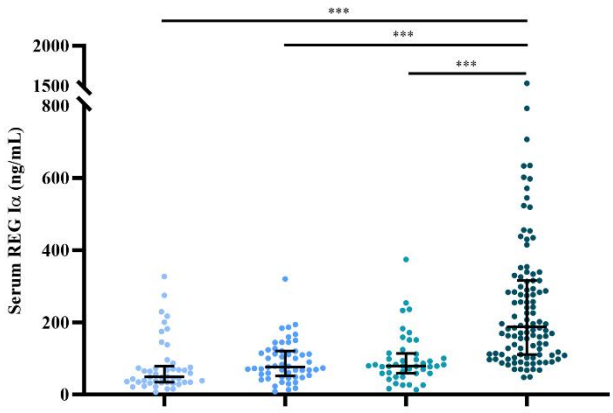

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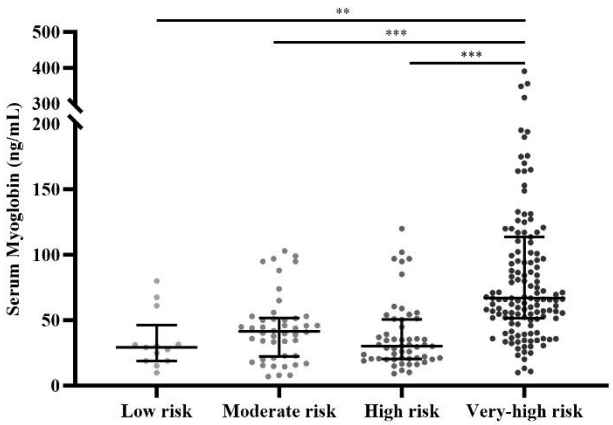
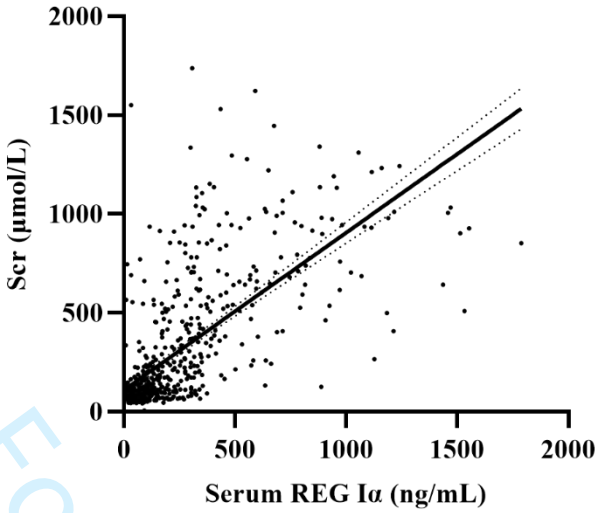


D2

Figure 1. Distribution of serum Cys-C, serum KIM-1, serum REG Iα, and serum Myoglobin in different groups.

A1, B1, C1, and D1 are in all participants: the CKD groups were classified in accordance with eGFR levels as described in methods. A1: Distribution of serum Cys-C. B1: Distribution of serum KIM-1. C1: Distribution of serum REG Iα. D1: Distribution of serum myoglobin. A2, B2, C2, and D2 are in patients with CKD: the patients were classified in accordance with 2012 KDIGO risk stratification as described in methods. A2: Distribution of serum Cys-C. B2: Distribution of serum KIM-1. C2: Distribution of serum REG Iα. D2: Distribution of serum Myoglobin. *: $P < 0.050$, **: $P < 0.010$, ***: $P < 0.001$. Tukey's multiple comparison tests were conducted to examine the differences in different groups. CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα.



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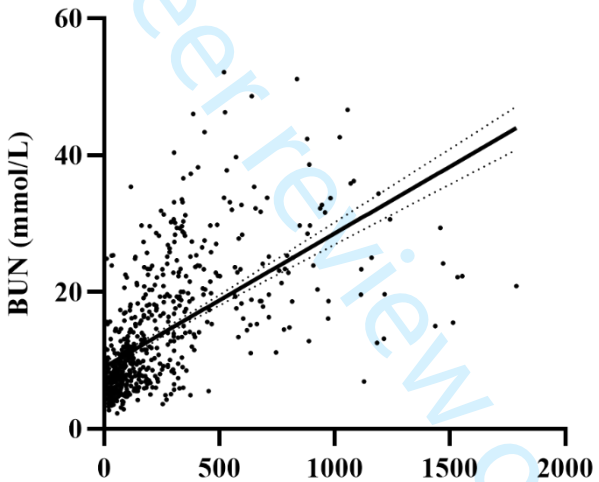

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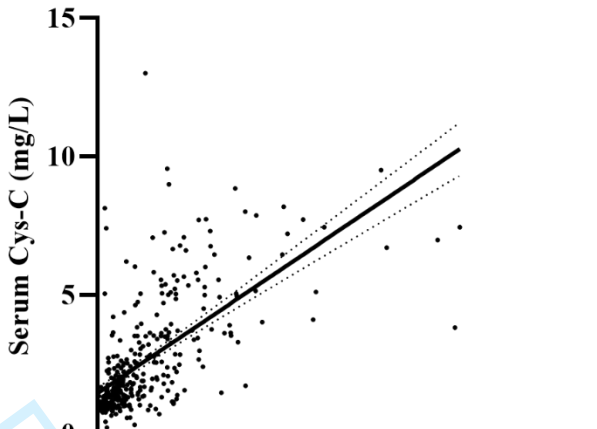

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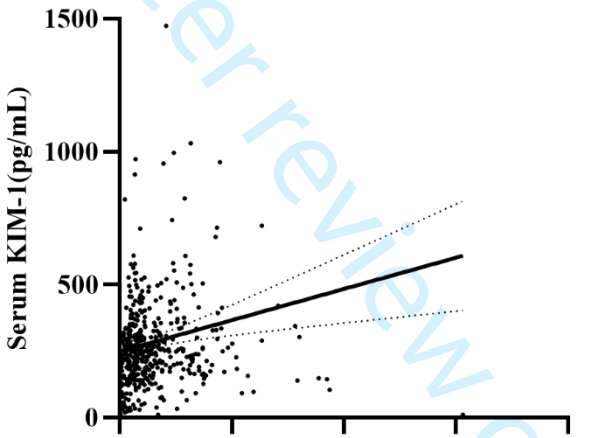

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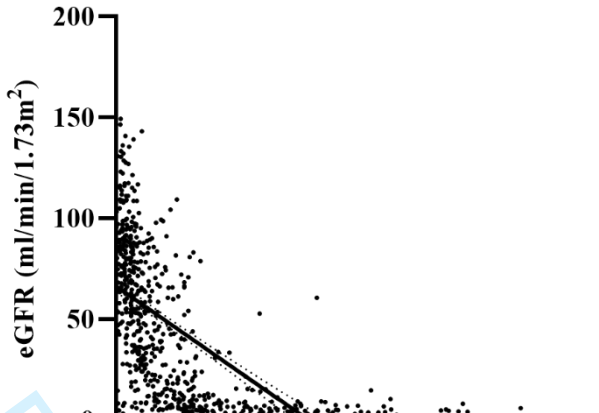


E

Figure 2. Correlations between serum REG Iα and different markers in all participants.

A: correlation between serum REG Iα and Scr (r = 0.753, $P < 0.001$), B: correlation between serum REG Iα and BUN (r = 0.733, $P < 0.001$), C: correlation between serum REG Iα and serum Cys-C (r = 0.678, $P < 0.001$), D: correlation between serum REG Iα and serum KIM-1 (r = 0.217, $P < 0.001$), E: correlation between serum REG Iα and eGFR (r = -0.789, $P < 0.001$). Scr: serum creatinine, BUN: blood urea nitrogen, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, eGFR: estimated glomerular filtration rate, REG Iα: regenerating protein Iα.

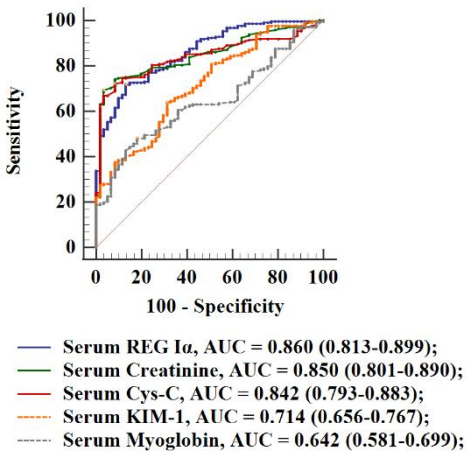


Figure 3. Ability of screening patients with CKD.

The AUC of serum REG Iα was 0.860 (95% CI: 0.813-0.899), and that of serum Creatinine was 0.850 (95% CI: 0.801-0.890), while that of serum Cys-C was 0.842 (95% CI: 0.793-0.883). Serum KIM-1 had an AUC of 0.714 (95% CI: 0.656-0.767), and serum Myoglobin had an AUC of 0.642 (95% CI: 0.581-0.699). The AUC of serum REG Iα was similar to serum Creatinine and serum Cys-C ($P > 0.050$), and was significantly higher than serum KIM-1 and serum Myoglobin ($P < 0.001$). DeLong tests were applied to analyze AUC differences between receiver operating characteristic curves. CKD: chronic kidney disease, AUC: area under the receiver operating characteristic curve, REG Iα: regenerating protein Iα, Cys-C: cystatin C, KIM-1: kidney injury molecular-1.

Supplementary material

Supplementary data Table 1. Correlations between serum REG Iα and different markers in all participants

Spearman Analyses (r)	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Age (1)	1.000													
Sex (2)	-0.060	1.000												
Diabetes (3)	0.367***	0.080**	1.000											
Hypertension (4)	0.262***	0.087**	0.236***	1.000										
CVD (5)	0.302***	0.024	0.125***	0.234***	1.000									
FBG (6)	0.258***	0.003	0.567***	0.050	0.062	1.000								
Scr (7)	0.037	0.294***	0.030	0.409***	0.098***	-0.159***	1.000							
BUN (8)	0.157***	0.170***	0.136***	0.442***	0.125***	-0.072**	0.845***	1.000						
UA (9)	-0.086**	0.267***	0.008	0.137***	0.036	-0.051	0.420***	0.379***	1.000					
eGFR (10)	-0.245***	-0.085**	-0.081**	-0.464***	-0.148***	0.123***	-0.927***	-0.856***	-0.328***	1.000				
Serum Cys-C (11)	-0.073	0.048	-0.237***	0.034***	0.079	-0.251***	0.811***	0.741***	0.336***	-0.826***	1.000			
Serum KIM-1 (12)	0.069	0.034	0.141***	0.078	0.031	0.105**	0.091**	0.121***	-0.076	-0.111**	0.111**	1.000		
Serum Myoglobin (13)	0.138***	0.117***	0.098**	0.229***	0.153***	0.024	0.648***	0.606***	0.210***	-0.661***	0.492***	0.066	1.000	

Serum REG Iα (14)	0.154***	0.059	0.073**	0.369***	0.099***	-0.134***	0.753***	0.733***	0.275***	-0.785***	0.678***	0.217***	0.565***	1.000

**: P < 0.050, ***: P < 0.001. CVD: cardiovascular disease, FBG: fast blood glucose, Scr: serum creatinine, BUN: blood urea nitrogen, UA: serum uric acid, eGFR: estimated glomerular filtration rate, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Iα: regenerating protein Iα.

Supplementary data Table 2. Distribution of serum Cys-C, serum KIM-1, serum REG Ⅰα and serum Myoglobin in KDIGO risk stratification.

KDIGO Risk Stratification Groups	**Low Risk (18.00%)**	**Moderate Risk (20.70%)**	**High Risk (17. 20%)**	**Very-high Risk (44.10%)**
Serum Cys-C (mg/L)	1.23 (1.01-1.43)	1.22 (1.05-1.45)	1.54 (1.17-1.79)	2.57 (1.94-3.53)
Serum KIM-1 (pg/mL)	255.77 (211.33-279.86)	262.46 (205.21-312.24)	275.72 (227.08-377.76)	247.28 (142.72-417.99)
Serum Myoglobin (ng/mL)	29.30 (18.97-46.25)	41.50 (22.39-51.82)	30.25 (20.60-50.52)	67.00 (51.70-113.68)
Serum REG Ⅰα (ng/mL)	48.86 (34.18-78.28)	76.05 (51.09-120.54)	79.18 (58.64-113.70)	184.38 (108.81-314.71)

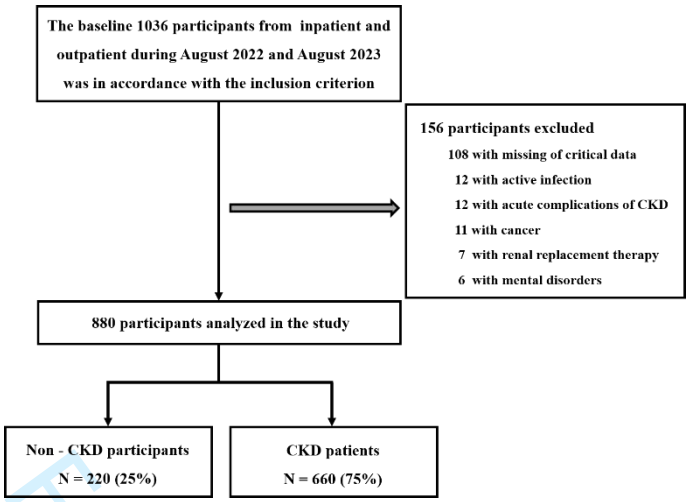
Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ⅰα: regenerating protein Ⅰα, KDIGO: kidney disease improving global outcomes.

The data were presented in quartiles.

Supplementary data Table 3. Ability of different biomarkers to screen patients with CKD.

Variables	AUC (95% CI)	Cutoff value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Ability of screening patients with CKD in all participants						
Serum REG Iα (ng/mL)	0.860 (0.813-0.899)	70.82	71.63	86.89	41.30	46.85
Serum Creatinine (μmol/L)	0.850 (0.801-0.890)	88.00	69.23	96.72	72.33	47.90
Serum Cys-C (mg/L)	0.842 (0.793-0.883)	1.33	72.12	91.80	55.57	49.09
Serum KIM-1 (pg/mL)	0.714 (0.656-0.767) ***	232.98	63.94	68.85	28.93	35.09
Serum Myoglobin (ng/mL)	0.642 (0.581-0.699) ***	59.70	48.08	81.97	33.50	30.72
Ability of screening high and very-high risk patients according to KDIGO risk stratification in CKD participants						
Serum REG Iα (ng/mL)	0.769 (0.712-0.819)	76.05	82.80	62.63	47.38	69.32
Serum Cys-C (mg/L)	0.865 (0.817-0.904) ***	1.63	75.16	92.93	80.65	70.00
Serum KIM-1 (pg/mL)	0.528 (0.465-0.590) ***	327.10	36.94	87.88	51.69	46.49

***: Comparing with serum REG Iα, P < 0.001. DeLong tests were applied to analyze AUC differences between receiver operating characteristic curves. CKD: chronic kidney disease, KDIGO: kidney disease improving global outcomes, Cys-C: cystatin C, KIM-1: kidney injury molecular-1, REG Ⅰα: regenerating protein Ⅰα. AUC: area under the receiver operating characteristic curve, PPV: positive predictive value, NPV: negative predictive value.



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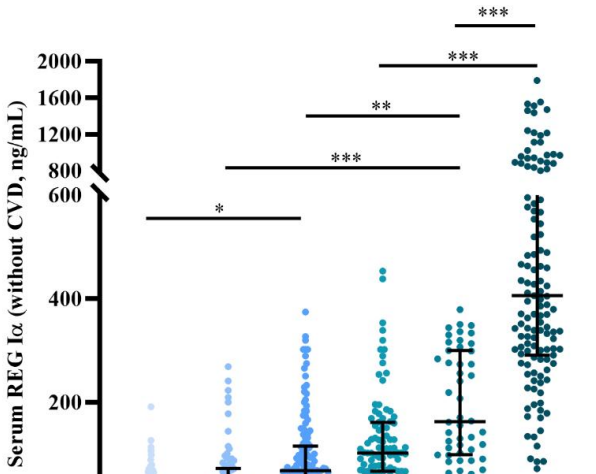
In the 1036 inpatient and outpatient participants, 880 were eligible for inclusion. Based on the exclusion criterion, participants were excluded because of missing critical data (n=108), active infection (n=12), acute complications of CKD (n=12), cancer (n=11), renal replacement therapy (n=7), and mental disorders (n=6). CKD: chronic kidney disease.



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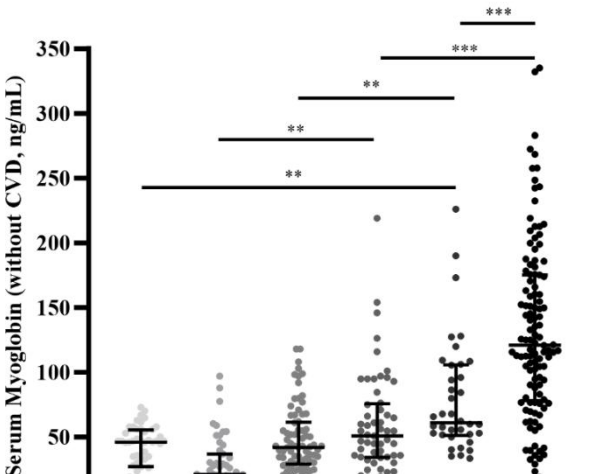

B

Supplementary data Figure 2. Distribution of serum REG Iα and Myoglobin in non-CKD and different CKD groups, which participants without CVD.

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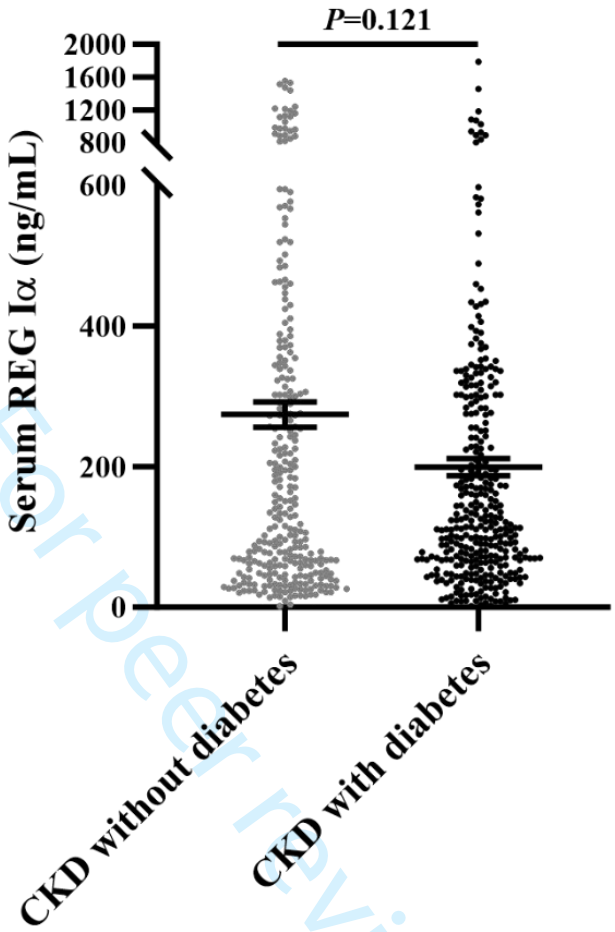

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