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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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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 This is a cross-sectional study.

Setting The study was 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 males, 50.45%) and 660 patients with CKD (366 males, 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 the CKD group (125.54 (60.28–303.39) ng/mL) compared with those in the 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, cystatin C (Cys-C), and kidney injury molecule 1 (KIM-1), while a negative correlation was identified with eGFR. After adjusting for sex, diabetes, hypertension and fasting blood glucose, 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 categorised 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 94.30% and a negative predictive value 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 outperformed 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 closely associated with kidney function. REG Iα may serve as a promising biomarker for CKD detection and risk stratification.

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

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study used robust logistic regression models to adjust for confounding factors and analyse the relationship between serum regenerating protein Iα (REG Iα) and kidney function.
⇒ This is the first study to apply Kidney Disease: Improving Global Outcomes risk stratification to explore the potential association between serum REG Iα and the risk of chronic kidney disease (CKD) progression.
⇒ The DeLong test was applied to statistically compare areas under the receiver operating characteristic curve values among the biomarkers of CKD, enhancing the reliability of diagnostic performance assessments.

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 characterised by a loss of approximately 90% of kidney function, rendering long-term survival without renal replacement therapy impossible.[2] The high prevalence, low detection rate, severe clinical outcomes and substantial economic 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), estimated 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 molecule 1 (KIM-1) and β2-microglobulin, have demonstrated the potential ability to enhance 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 that 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), consistent with the findings of Sobajima and others.[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, assess 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) patients with CKD: age >18 years and diagnosed with CKD in accordance with the 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 and (9) mental disorders.

eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration 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 guidelines.[26 27] Subgroup analyses were conducted among patients with CKD 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 generalisability of the study.

Data collection and quality assessment

Baseline demographic data were collected using standardised questionnaires. All participants underwent 12-hour fasting and took about 3 mL of the peripheral blood samples. Blood samples were centrifuged at 3500 rpm for 15 min, and the upper serum was collected within 6 hours. Serum samples were immediately stored at −80°C for subsequent analyses. The following clinical biochemical parameters were extracted from the clinical laboratory of the Hospital: 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 summarised as mean±SD for normally distributed data or as median with 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 nonparametric 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 χ^2 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 three or more groups, thereby avoiding the issue of multiple comparisons.

Spearman's rank correlation analyses and ordinal logistic regression were used to measure the associations between serum REG Iα and other biomarkers of kidney function. Multivariate logistic regression analysis was used to identify the independent factors of kidney dysfunction. The study included age, BUN, UA, serum myoglobin, serum Cys-C, serum KIM-1/100 and serum REG Iα/100 into an 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 analyse 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 to evaluate accuracy using sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and accuracy, and applied the DeLong test to statistically analyse AUC differences between receiver operating characteristic (ROC) 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 patients with CKD (online supplemental data figure 1). Significant differences were observed between patients with CKD and non-CKD participants in terms of age, complication diseases (diabetes and hypertension), 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 patients with CKD (125.54 (60.28, 303.39) ng/mL) compared with 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 the non-CKD and CKD groups (G2 to G5, p<0.010, figure 1: B1). Further analyses confirmed that cardiovascular disease (online supplemental data figure 2) did not influence the distribution of serum REG Iα and myoglobin levels. Diabetes had no influence on serum REG Iα in patients with CKD (online supplemental data figure 3).

Table 1 Clinical characteristics of the study population at baseline examination

	Non-CKD participants	Patients with CKD	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 (yes, %)	44 (20.00)	149 (22.58)	0.453
Laboratory measurements			
Fast blood glucose (mmol/L)	5.45 (4.99–6.67)	5.34 (4.57–7.11)	0.008
Serum creatinine (μmol/L)	64.00 (55.25–76.00)	126.00 (83.00–418.50)	<0.001
Blood urea nitrogen (mmol/L)	5.20 (4.30–6.28)	10.30 (7.00–18.60)	<0.001
Serum uric acid (μmol/L)	301.00 (256.00–365.25)	354.00 (290.00–443.00)	<0.001
Estimated glomerular filtration rate (mL/min/1.73 m^2)	102.37 (95.73–112.36)	44.03 (10.96–77.09)	<0.001
Serum cystatin C (mg/L)	1.10 (0.99–1.24)	1.79 (1.23–3.50)	<0.001
Serum kidney injury molecule 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 (ng/mL)	24.62 (14.09–37.32)	125.54 (60.28–303.39)	<0.001

Data are 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. χ^2 or Fisher's exact tests were used for categorical variables for two group comparisons.
CKD, chronic kidney disease.

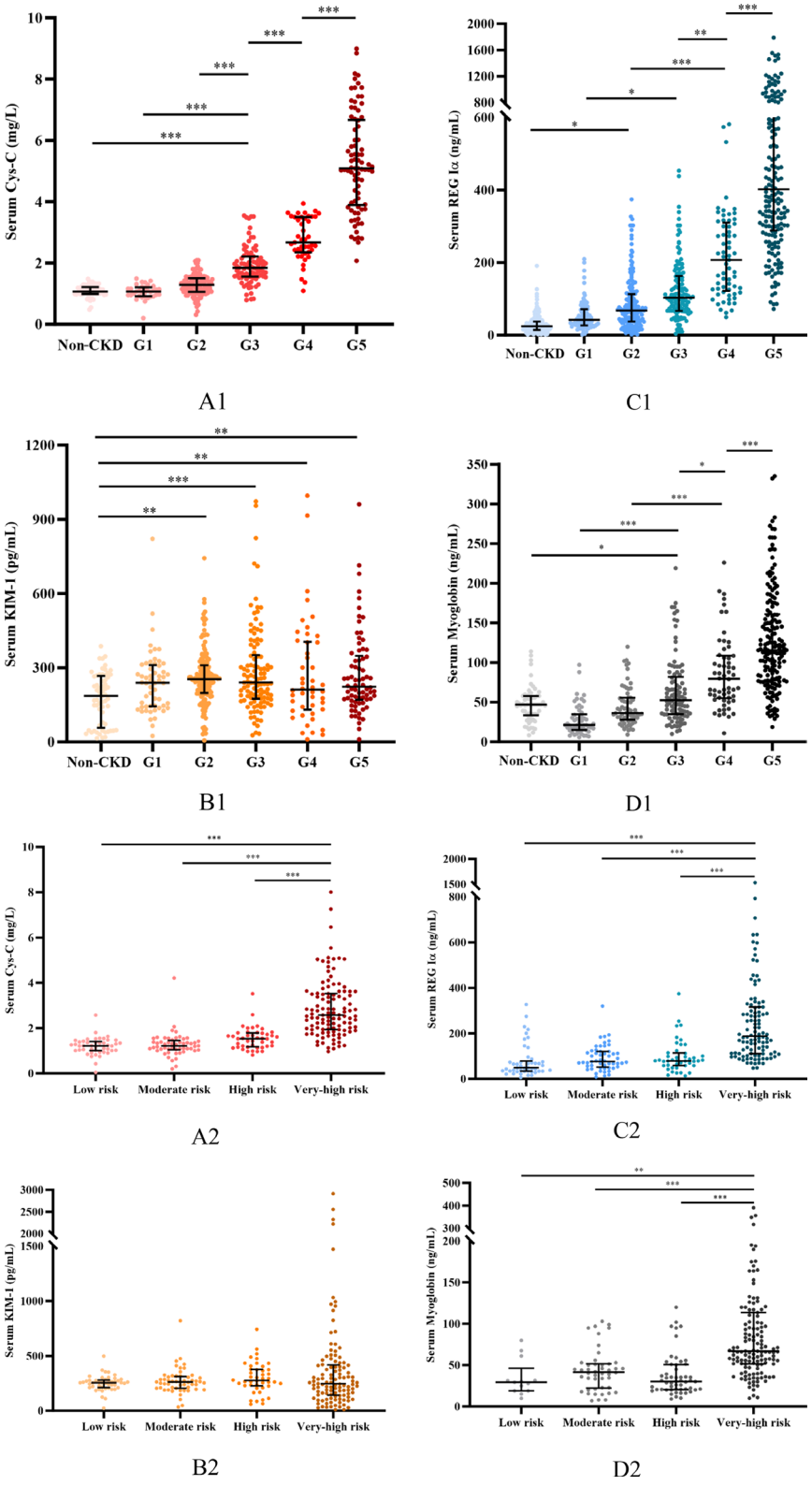

Figure 1 Distribution of serum Cys-C, serum KIM-1, serum REG Iα and serum myoglobin in different groups. A1, B1, C1 and D1 show distributions in all participants: the CKD groups were classified in accordance with eGFR levels as described in the Methods section. A1: Distribution of serum Cys-C. B1: Distribution of serum KIM-1. C1: Distribution of serum REG Iα. D1: Distribution of serum myoglobin. A2, B2, C2 and D2 show distributions in patients with CKD: the patients were classified in accordance with KDIGO 2012 risk stratification as described in the Methods section. 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 and ***p<0.001. Tukey's multiple comparison tests were conducted to examine the differences in different groups. CKD, chronic kidney disease; Cys-C, cystatin C; KDIGO, Kidney Disease Improving Global Outcomes; KIM-1, kidney injury molecule 1; REG Iα, regenerating protein Iα.

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 correlation 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). A significant 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 online supplemental 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 were significantly associated with eGFR (OR=1.737, 95% CI: 1.263 to 2.388, p=0.001, table 2).

Subgroup analysis in patients with CKD

To explore the relationship between serum REG Iα levels and CKD progression risk, 256 patients with CKD were included in the sub-research and classified by eGFR and UACR levels in accordance with KDIGO risk stratification guidelines.[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%, online supplemental 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-risk CKD (OR=1.799, 95% CI: 1.088 to 2.975, p=0.022, table 2).

Ability of serum REG Iα in screening patients with kidney dysfunction

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 online supplemental data table 3). Serum REG Iα demonstrated an AUC of 0.860 (95% CI: 0.813 to 0.899) for detecting CKD compared with Scr (0.850, 95% CI: 0.801 to 0.890) and serum Cys-C (0.842, 95% CI: 0.793 to 0.883). At a cut-off value of 70.82 ng/mL, serum REG Iα had a sensitivity of 71.63%, specificity of 86.89%, PPV of 94.30% and NPV of 46.85%. Serum KIM-1 showed a lower AUC than serum REG Iα, measuring 0.714 (95% CI: 0.656 to 0.767, p<0.001). Serum myoglobin had the lowest AUC among the five biomarkers, measuring 0.642 (95% CI: 0.581 to 0.699, p<0.001).

For distinguishing high- and very-high-risk CKD, serum REG Iα demonstrated superior performance compared with serum KIM-1 (AUC=0.769 (0.712–0.819) vs 0.528 (0.465–0.590), p<0.010, online supplemental 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 cut-off 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 is the first study to systematically evaluate serum REG Iα levels in a broad CKD population and confirm its upregulation in patients with CKD. 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 the pancreas and was identified as PSP due to its role in inhibiting the formation of calcium carbonate stones 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 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. 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 CKD (group G1 and group G2). There was also a gradual increase in patients with medium- to end-stage CKD (group G3 to group G5). The possible reason was as follows: in the early stages of kidney injury, compensatory glomerular hypertrophy with a 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/1.73 m^2) and even appearing as a transient decrease. With progression to medium and end stages of CKD (eGFR <60 mL/min/1.73 m^2), the glomerular basement membrane

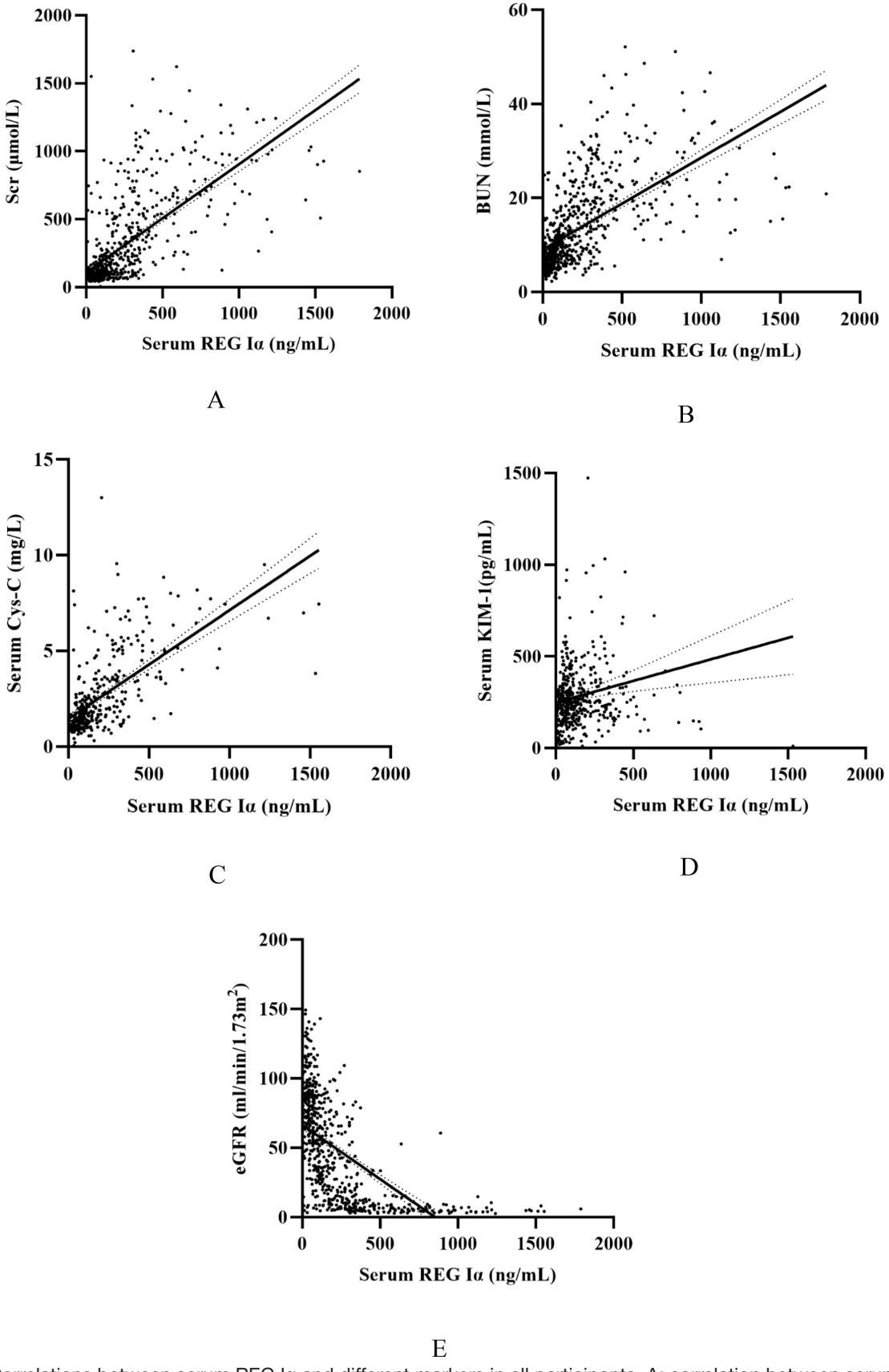

Figure 2 Correlations between serum REG Iα and different markers in all participants. A: correlation between serum REG Iα and Scr (r=0.753, p<0.001), B: correlation between serum REG Iα and BUN (r=0.733, p<0.001), C: correlation between serum REG Iα and serum Cys-C (r=0.678, p<0.001), D: correlation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

Table 2 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‡	0.050	1.020 (1.001 to 1.041)	0.009	0.966 (0.942 to 0.992)
BUN§	<0.001	1.266 (1.165 to 1.376)	<0.001	1.440 (1.216 to 1.706)
UA¶	0.337	1.001 (0.999 to 1.004)	0.085	1.003 (1.000 to 1.007)
Serum myoglobin**	0.165	1.005 (0.998 to 1.013)	0.148	1.136 (0.897 to 1.559)
Serum Cys-C††	<0.001	6.784 (4.016 to 11.460)	0.071	1.853 (0.949 to 3.620)
Serum KIM-1/100‡‡	0.133	1.069 (0.980 to 1.167)	0.122	1.243 (0.943 to 1.639)
Serum REG Iα/100**	0.001	1.737 (1.263 to 2.388)	0.022	1.799 (1.088 to 2.975)

* The ordinal multiple logistic regression shows 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 and 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.
‡ years.
§ mmol/L.
¶ μmol/L.
** ng/mL.
†† mg/L.
‡‡ pg/mL.
BUN, blood urea nitrogen; CKD, chronic kidney disease; Cys-C, cystatin C; eGFR, estimated glomerular filtration rate; FBG, fast blood glucose; KDIGO, Kidney Disease Improving Global Outcomes; KIM-1, kidney injury molecule 1; REG Iα, regenerating protein Iα; UA, uric acid.

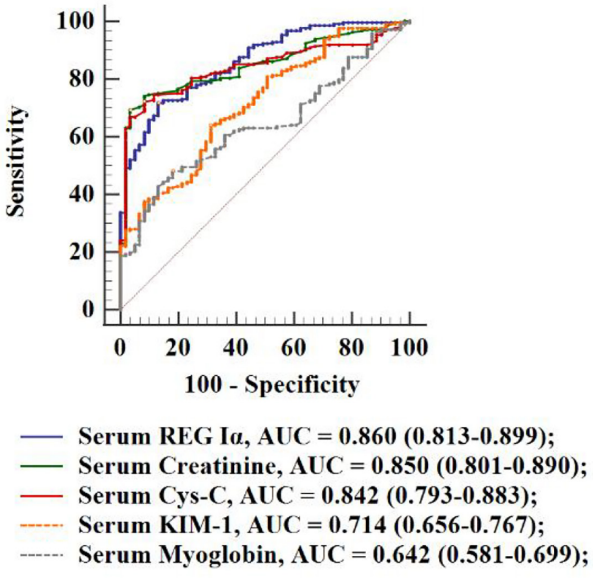

Figure 3 Ability of screening patients with CKD. The AUC of serum REG Iα was 0.860 (95% CI: 0.813 to 0.899) and that of Scr was 0.850 (95% CI: 0.801 to 0.890), while that of serum Cys-C was 0.842 (95% CI: 0.793 to 0.883). Serum KIM-1 had an AUC of 0.714 (95% CI: 0.656 to 0.767), and serum myoglobin had an AUC of 0.642 (95% CI: 0.581 to 0.699). The AUC of serum REG Iα was similar to Scr and serum Cys-C (p>0.050) and was significantly higher than serum KIM-1 and serum myoglobin (p<0.001). The DeLong tests were applied to analyse AUC differences between receiver operating characteristic curves. AUC, area under the receiver operating characteristic curve; CKD, chronic kidney disease; Cys-C, cystatin C; KIM-1, kidney injury molecule 1; REG Iα, regenerating protein Iα.

thickened and led to a significant decline in glomerular filtration function, ultimately causing protein 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 behaviour of serum REG Iα, compared with low-molecular-weight protein, highlights its dual role: accumulation due to reduced glomerular clearance and potentially increased production in response to kidney injury. Unlike myoglobin, which remains stable in early-stage CKD, 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 aetiology 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 antiapoptosis 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 synthesised in the pancreas and released into 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 in 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 focuses on glomerular filtration capacity, which is characterised 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 the 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 with 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 with serum KIM-1, the REG Iα had several advantages as follows. This study 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 patients with CKD. 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 patients with CKD. These features collectively underscore its potential as a potential biomarker for CKD.

In this study, serum REG Iα was strongly correlated with Scr and Cys-C and had a similar performance to Scr 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 Scr and Cys-C in detecting ultra-early stage of kidney dysfunction. A 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. It also emerged as an independent risk factor for patients with CKD categorised 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 the 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 aetiology of CKD. Globally, diabetes and hypertension are recognised 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 aetiology in our study.

There are some limitations in this study. First, the cross-sectional design precluded causal inference. Due to the challenges of obtaining detailed data on renal biopsy, our team has endeavoured to conduct a pre-subgroup analysis within the available constraints. We found that CKD etiologies (eg, IgA nephropathy, membranous nephropathy and DKD) have no effect on serum REG levels. To enhance causality and generalisability, 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. Fourth, 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 patients with CKD 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 standardised 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 patients with CKD 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 patients with high-risk CKD.

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