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ORIGINAL ARTICLE

Plasma kidney injury molecule-1 (p-KIM-1) levels and deterioration of kidney function over 16 years

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ABSTRACT

Background. The kidney injury molecule-1 (KIM-1) has previously been associated with kidney function in rodents and humans. Yet its role as a predictive marker for future decline in kidney function has remained less clear.

Methods. At baseline (1991–1994), fasting plasma KIM-1 (p-KIM-1) was measured in 4739 participants of the population-based Malmö Diet and Cancer Study. Creatinine and cystatin C were used to calculate estimated glomerular filtration rate (eGFR) according to Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Collaboration 2012 creatinine–cystatin C equation at baseline and follow-up examination (2007–2012). Incident CKD was defined as an eGFR <60 mL/min/1.73 m^2 at follow-up.

Results. During a mean follow-up time of 16.6 years, high p-KIM-1 levels were associated with a greater decline in eGFR (quartile 1 −1.36 versus quartile 4 −1.54 mL/min/1.73 m^2; $P < 0.001$). In multivariate analyses, the risk for incident CKD at the follow-up examination was higher among participants with baseline p-KIM-1 levels in the highest quartile {odds ratio [OR] 1.45 [95% confidence interval (CI) 1.10–1.92]} compared with those within the lowest quartile. The relative impact of baseline p-KIM-1 on incidence of CKD [OR 1.20 (95% CI 1.08–1.33) per 1 standard deviation (SD) increase in p-KIM-1] was comparable to those of age and systolic blood pressure (SBP) [OR 1.55 (95% CI 1.38–1.74) and OR 1.21 (95% CI 1.09–1.35) per 1 SD increase, respectively]. Adding p-KIM-1 to a conventional risk model resulted in significantly improved C-statistics ($P = 0.04$) and reclassified 9% of the individuals into the correct risk direction (continuous net reclassification improvement $P = 0.02$). Furthermore, the risk for hospitalization due to impaired renal function increased with increasing baseline p-KIM-1 [hazard ratio per 1 SD 1.43; (95% CI 1.18–1.74)] during a mean follow-up time of 19.2 years.

Conclusion. Our results show that p-KIM-1 predicts the future decline of eGFR and risk of CKD in healthy middle-aged participants. Whether p-KIM-1 can be used to prioritize preventive action that needs to be further investigated.

Keywords: CKD, eGFR, kidney function, KIM-1, TIM-1

INTRODUCTION

The kidney injury molecule-1 (KIM-1), also known as T-cell immunoglobulin and mucin-domain-containing molecule-1 (TIM-1), was first proposed to have a role in restoration after a kidney injury almost 2 decades ago, when KIM-1 was found to be markedly upregulated in the proximal tubular cells of rats after ischaemic injury [1]. More recently, chronic expression of KIM-1 in renal epithelial cells of transgenic mice was observed to lead to tubular interstitial fibrosis and inflammation, whereas mice with a truncated form of KIM-1 were protected from fibrosis [2]. In the first human study, high urinary KIM-1 (u-KIM-1) levels and extensive expression of this protein were detected in biopsies from the proximal tubule of patients with acute tubular necrosis [3]. Furthermore, higher u-KIM-1 has been associated with an enhanced decline of estimated glomerular filtration rate (eGFR) in patients with type 1 (T1D) [4] and type 2 diabetes (T2D) [5], whereas lower levels have been associated with regression of microalbuminuria in T1D [6]. However, studies evaluating u-KIM-1 as a marker of diabetic nephropathy have been inconclusive [4–8]. Moreover, u-KIM-1 did not add significant additional independent prognostic information in a prediction model with known risk factors in studies of T1D and T2D patients [4, 5], nor did it associate with CKD progression in a recent study of the Chronic Renal Insufficiency Cohort after controlling for urinary albumin:creatinine ratio and eGFR [9]. Another recent study reported that higher levels of u-KIM-1 were associated with lower eGFR and greater albuminuria in pooled cross-sectional multivariate analyses of five racially and ethnically diverse cohorts from Sweden and the USA [10].

Concerning plasma KIM-1 (p-KIM-1), results from two controlled trails (ACCORD and VA NEPHRON-D) for early and advanced diabetic kidney disease (DKD) showed that higher p-KIM-1 levels were associated with a greater eGFR decline independent of known clinical risk factors [11]. Likewise, higher circulating levels were associated with deterioration of kidney function in T1D patients in the Joslin Kidney Study [12]. Importantly, that study investigated both u-KIM-1 and p-KIM-1 and observed that while high p-KIM-1 levels were associated with an increased risk of early progressive renal decline (independent of u-KIM-1), such an association was not seen for u-KIM-1 once p-KIM-1 was included in the analysis [12].

Taken together, although the current evidence indicates a role for KIM-1 in kidney function, the majority of studies so far have measured u-KIM-1 [3–6, 8–10] and less is known about the role of p-KIM-1 in kidney function [11–13]. Furthermore, whether p-KIM-1 may predict a longitudinal decline of kidney function in the generally healthy population has not been investigated. Therefore the aim of this study was to investigate if baseline levels of p-KIM-1 associate with longitudinal decline in kidney function, incidence of CKD or hospitalization due to renal impairment in the population-based Malmö Diet and Cancer Study (MDCS).

MATERIALS AND METHODS

MDCS

During 1991 and 1996, all men and women living in Malmö and born between 1923–45 and 1923–50, respectively, were invited to participate in the MDCS. The participation rate was 40.8% [14] and a detailed description of the cohort has been published elsewhere [15]. The study was approved by the ethical committee at Lund University (LU 51-90) and was carried out in accordance with the Helsinki Declaration. Written informed consent was provided by all the participants.

For this study, we included 4739 participants that provided fasting plasma samples at baseline from the MDCS Cardiovascular Cohort (MDCS-CC, $N = 6103$), which is a randomly selected subcohort of MDCS that underwent additional phenotyping between 1991 and 1994. Participants with missing data for smoking ($n = 6$), low-density lipoprotein (LDL) ($n = 6$), creatinine ($n = 54$) or cystatin C ($n = 261$) were excluded, leading to a study population of 4412 participants. Between 2007 and 2012, a total of 3734 MDCS-CC participants of those that were alive and had not emigrated from Sweden ($n = 4924$), attended a follow-up examination, which has been described previously [16]. Of these, 2799 participants had data on baseline p-KIM-1 and kidney function measures (Figure 1).

Clinical examination and assays

All participants underwent a physical examination and anthropometric measurements were obtained by trained nurses during the baseline examination. Systolic and diastolic blood pressure (SBP and DBP) were measured. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Questions concerning socio-economic status, lifestyle factors and medical history were assessed via a self-administrated questionnaire [15]. Fasting blood samples were drawn and immediately frozen to −80°C and stored in a biobank [17]. Creatinine (μmol/L) was measured in plasma and analysed with the Jaffé method and traceable to the international standardization with isotope dilution mass spectrometry (IDMS). Cystatin C was measured using a particle-enhanced immunonephelometric assay (N Latex Cystatin; Dade Behring, Deerfield, IL, USA). The values of cystatin C were not standardized because they were analysed before the introduction of the world calibrator in 2010. The reference values for the method were 0.53–0.95 mg/L. Calculation of eGFR was done according to the previously reported Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) creatinine–cystatin C equation [18].

Following similar approaches as at baseline, anthropometric characteristics, SBP and DBP were measured during the follow-up examination (2007–12). Furthermore, fasting plasma glucose (mmol/L), creatinine (μmol/L) and cystatin C (mg/L) were measured using the same analytical methods as at baseline.

Baseline levels of p-KIM-1 were analysed by the Proximity Extension Assay technique using the Proseek Multiplex CVD$^{96\times96}$ reagents kit (Olink Bioscience, Uppsala, Sweden) at the Clinical Biomarkers Facility, Science for Life Laboratory, Uppsala. Oligonucleotide-labelled antibody probe pairs were allowed to bind to their respective targets present in the plasma sample and addition of a DNA polymerase led to an extension and joining of the two oligonucleotides and formation of a polymerase chain reaction (PCR) template. Universal primers were used to pre-amplify the DNA templates in parallel. Finally, the individual DNA sequences were detected and quantified using specific primers by a microfluidic real-time quantitative PCR chip (96.96, Dynamic Array IFC, Fluidigm Biomark). The chip was run with a Biomark HD instrument. The coefficient of variation for intra-assay variation (within-run) and interassay variation (between-run) for KIM-1 was 11% and 9%. Data analysis was performed by a preprocessing normalization procedure using Olink Wizard for GenEx (Multid Analyses, Göteborg, Sweden). All data are presented as normalized protein expression. General calibrator curves to calculate the approximate concentrations as well as technical information about the assays are available on the Olink homepage (http://www.olink.com).

Renal outcomes

CKD at follow-up examination was defined as an eGFR <60 mL/min/1.73 m^2 based on one measurement of creatinine and cystatin C at the follow-up examination. We excluded all participants with prevalent CKD at baseline ($n = 34$) from the analysis of CKD incidence at follow-up.

We linked the MDCS-CC cohort to the Swedish patient register to obtain information on hospitalization due to impaired renal function (hiRF). The Swedish patient register covers all hospitalizations in Sweden since 1987 and hospital outpatient visits from 2001 onwards. The register has been previously described and validated for outcome classification [19]. The follow-up of all participants was performed until the occurrence of hiRF, emigration from Sweden or 31 December 2013. We defined hiRF as 585–586 according to the International Classification of Diseases, Revision 9 (ICD-9) and N18 and N19

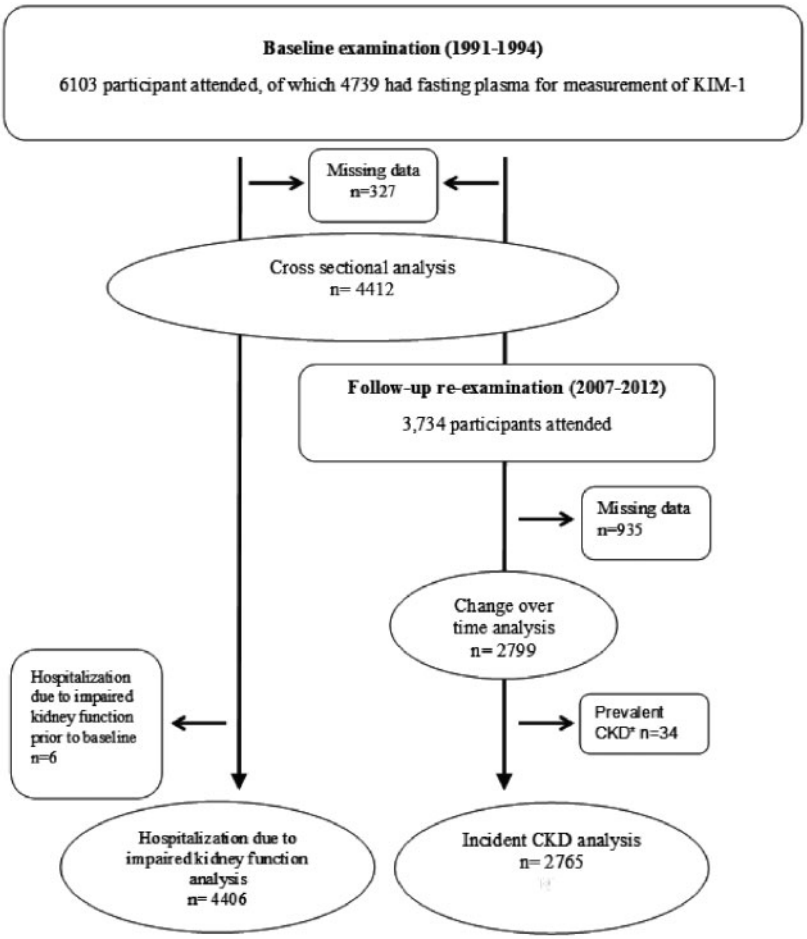

FIGURE 1: Participants of the MDCS included in the cross-sectional and longitudinal association analyses for p-KIM-1 levels and kidney function. *at baseline eGFR $<$ 60 mL/min/1.73m^2.

according to ICD, revision 10. We additionally differentiated between hiRF as the main diagnosis, when registered as the first diagnosis in the patient record (90 cases), and hiRF as a contributing diagnosis, when the above-mentioned ICD codes were registered at a position other than the first (172 cases in total). For the analysis of incident hiRF, we excluded all participants with impaired kidney function at baseline ($n = 6$).

The CKD diagnoses were validated in a two-step quality process by two experienced specialists in nephrology who independently reviewed patient records and laboratory data in 100 randomly selected patients following the ICD-9 and ICD-10 codes for CKD. The validation showed that 94% of the patients had a correct diagnosis of CKD. Further details were published earlier [20].

Statistics

We analysed the baseline levels of p-KIM-1 per 1 standard deviation (SD) increment of the log value. In addition, we categorized the study sample according to p-KIM-1 levels into sex-specific equal quartiles. We tested the association between p-KIM-1 and baseline characteristics using a general linear model (GLM) for continuous variables adjusted for age and sex and a chi-square test for categorical variables. Furthermore, we tested the relationship between p-KIM-1 and the change in eGFR from the baseline to the follow-up examination, for which we calculated the annual change in eGFR by subtracting the baseline value from the follow-up value and dividing it by the follow-up time in years to account for the different lengths of follow-up.

Logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) for incidence of CKD (eGFR at follow-up $<$60 mL/min/1.73 m^2). For the basic model we included age, sex and follow-up time as covariates. The net reclassification improvement (NRI) [21] was calculated using the nri STATA command for the package idi (http://personalpages.manchester.ac.uk/staff/mark.lunt). The receiver operating characteristics curves for incident CKD were generated and the areas under the curves (AUCs) of covariate-adjusted models with and without inclusion of p-KIM-1 were calculated using the roccomp command in STATA to compare predictive accuracy.

Cox proportional hazard (PH) regression was used to estimate the hazard ratio (HR) and 95% CI for the incidence of CKD. The PH assumption was tested using the Schoenfeld residuals (estat STATA command) and graphical (stphplot STATA command) assessment. The hazard function was graphically examined by plotting the Kaplan–Meier failure function (sts graph STATA command) according to quartiles of

Table 1. Clinical characteristics of the MDCS-CC participants at baseline examination (1991–1996) according to quartiles of p-KIM-1

Clinical characteristics	All	Q1	Q2	Q3	Q4	P-value
N	4412	1103	1103	1103	1103	
p-KIM-1[a], mean (range)						
Males		4.22 (2.22–4.67)	4.93 (4.67–5.19)	5.42 (5.19–5.66)	6.22 (5.67–10.44)	
Females		4.19 (2.34–4.63)	4.87 (4.63–5.09)	5.32 (5.10–5.56)	6.06 (5.57–8.88)	
Male (%)	39.3	39.3	39.3	39.3	39.3	1.00
Age (years)	57.434 (5.945)	55.040 (5.811)	57.017 (5.836)	57.945 (5.774)	59.733 (5.370)	0.006
Height (cm)	168.813 (8.907)	169.595 (8.722)	169.059 (9.044)	168.461 (8.921)	168.109 (8.877)	<0.0001
Weight (kg)	73.100 (13.368)	72.213 (12.515)	72.225 (13.066)	73.378 (13.246)	74.582 (14.446)	<0.0001
BMI (kg/m^2)	25.590 (3.912)	25.038 (3.504)	25.202 (3.797)	25.806 (3.974)	26.312 (4.214)	<0.0001
Waist (cm)	83.230 (12.705)	81.487 (11.828)	82.121 (11.852)	83.659 (12.902)	85.651 (13.745)	<0.0001
SBP (mmHg)	140.804 (18.820)	135.410 (17.076)	138.030 (17.458)	142.550 (18.826)	147.230 (19.640)	<0.0001
DBP (mmHg)	86.653 (9.287)	85.150 (8.495)	85.150 (8.894)	87.300 (9.488)	89.020 (9.663)	<0.0001
Fasting glucose (mmol/L)	5.124 (1.273)	4.892 (0.781)	5.008 (0.961)	5.132 (1.071)	5.465 (1.902)	<0.0001
HbA1c (%)	4.885 (0.705)	4.683 (0.484)	4.821 (0.568)	4.885 (0.603)	5.150 (0.976)	<0.0001
Total cholesterol (mmol/L)	6.144 (1.069)	5.866 (1.018)	6.041 (1.000)	6.214 (1.055)	6.454 (1.110)	<0.0001
HDL (mmol/L)	1.394 (0.374)	1.399 (0.357)	1.398 (0.353)	1.389 (0.374)	1.389 (0.408)	0.266
LDL (mmol/L)	4.156 (0.976)	3.948 (0.929)	4.076 (0.914)	4.216 (0.980)	4.386 (1.024)	<0.0001
TG (mmol/L)	1.307 (0.638)	1.141 (0.535)	1.246 (0.603)	1.347 (0.621)	1.493 (0.724)	<0.0001
Cystatin C (mg/L)	0.775 (0.151)	0.741 (0.119)	0.761 (0.127)	0.777 (0.135)	0.820 (0.197)	<0.0001
Plasma creatinine (μmol/L)	84.483 (16.329)	84.700 (14.804)	83.270 (13.649)	83.990 (15.443)	85.970 (20.479)	0.952
eGFR[b] (mL/min/1.73 m^2)	89.064 (13.568)	91.624 (12.623)	90.496 (13.015)	88.823 (13.209)	85.315 (14.524)	0.0007
AHT (%)	16.7	11.2	13.8	17.1	24.8	<0.001
Current smoking (%)	25.9	20.4	22.8	26.6	33.9	<0.001

Data are shown as mean (SD).
P-value from a general linear regression adjusted for age and sex (continues) or chi-square-test (categorical variables).
[a]Log transformed.
[b]The eGFR is based on CKD-EPI creatinine–cystatin C equation 2012 [18] (mL/min/1.73 m^2).
AHT, anti-hypertensive treatment; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; LDL, low density lipoprotein; MDCS-CC, Malmö Diet and Cancer Study-Cardiovascular Cohort; SBP, systolic blood pressure; TG, triglycerides.

p-KIM-1. Given the known relationship between age and eGFR, age was used as the underlying time variable (i.e. to define time to event) in the Cox regression model. In the basic model we included sex as a covariate.

The final model for both Cox and logistic regression was adjusted for further risk factors of CKD: BMI, eGFR, fasting glucose, SBP, smoking status (current, former or never smokers) and use of anti-hypertensive treatment (AHT) (yes/no) at baseline. P-values ≤ 0.05 were considered statistically significant. SPSS (version 21, IBM, Armonk, NY, USA) and STATA version 13 (StataCorp, College Station, TX, USA) were used for analysis.

RESULTS

Baseline characteristics

In total, 4412 participants were included in the study. High baseline values of p-KIM-1 were significantly associated with lower baseline eGFR, female sex, older age, use of AHT, current smoking and decreased height (Table 1). Furthermore, we observed positive associations between p-KIM-1 and several cardiometabolic characteristics and therefore additionally stepwise adjusted for all the tested baseline variables, yet none of them markedly changed the results (Supplementary data, Table S1).

Longitudinal changes

At the follow-up examination, the highest quartile of baseline p-KIM-1 was associated with a greater decline in total cholesterol and LDL ($P < 0.001$) but not with further anthropometric or clinical measurements. The mean decline in eGFR at follow-up was 23.92 mL/min/1.73 m^2 (SD 13.74). High baseline p-KIM-1 associated with a greater annual decline in eGFR [quartile 1 (Q1) −1.36 versus quartile 4 (Q4) −1.54 mL/min/1.73; $P < 0.001$] and an increase in plasma creatinine ($P < 0.001$) and plasma cystatin C ($P < 0.001$) after adjusting for age, sex, follow-up time and baseline levels of eGFR, creatinine or cystatin C, respectively. When the linear regression model was further adjusted for known risk factors of kidney dysfunction (BMI, SBP, fasting glucose, AHT and smoking), the higher baseline p-KIM-1 remained associated with a greater annual decline in eGFR (P-trend = 0.01) and an increase in plasma creatinine (P-trend = 0.01) and plasma cystatin C (P-trend = 0.01). (Table 2 and Figure 2A).

Incidence of CKD

CKD occurred in 882 participants, corresponding to 19.2 cases/1000 person-years throughout a follow-up time of 13.3–20.2 years. We observed a 70% increased incidence of CKD at the follow-up examination in participants with high levels (Q4) of p-KIM-1 at baseline compared with participants with low levels (Q1) in the basic model [OR 1.70 (95% CI 1.29–2.22)]. Although adding baseline eGFR, BMI, SBP, fasting glucose, AHT and smoking to the logistic regression model attenuated the risk increase, it clearly remained increased [OR 1.45 (95% CI 1.10–1.92)] (Figure 2B). When comparing a logistic regression model adjusted for known CKD risk factors (age, sex,

Table 2. Change in clinical characteristics including markers of kidney function between baseline and follow-up examination in 2799 participants of the MDCS-CC according to quartiles of p-KIM-1 at baseline

Clinical characteristics	Q1	Q2	Q3	Q4	P-value
Weight (kg)	2.357 ± 0.289	2.840 ± 0.284	2.183 ± 0.285	2.000 ± 0.292	0.183
BMI (kg/m^2)	1.351 ± 0.103	1.505 ± 0.101	1.317 ± 0.102	1.254 ± 0.104	0.300
Waist (cm)	9.206 ± 0.293	9.372 ± 0.288	9.170 ± 0.289	9.302 ± 0.289	0.955
SBP (mmHg)	5.226 ± 0.689	5.052 ± 0.674	4.992 ± 0.676	3.855 ± 0.693	0.187
DBP (mmHg)	−3.011 ± 0.379	−2.645 ± 0.372	−2.446 ± 0.372	−3.783 ± 0.382	0.229
Fasting glucose (mmol/L)	0.563 ± 0.046	0.642 ± 0.045	0.646 ± 0.045	0.669 ± 0.046	0.124
Total cholesterol (mmol/L)	−0.879 ± 0.038	−0.866 ± 0.037	−0.962 ± 0.037	−1.146 ± 0.038	<0.001
HDL (mmol/L)	0.023 ± 0.011	0.013 ± 0.011	0.012 ± 0.011	−0.004 ± 0.011	0.101
LDL (mmol/L)	−0.774 ± 0.034	−0.763 ± 0.034	−0.858 ± 0.034	−1.035 ± 0.034	<0.001
TG (mmol/L)	−0.204 ± 0.019	−0.162 ± 0.018	−0.174 ± 0.018	−0.188 ± 0.019	0.694
Cystatin C (mg/L)	0.372 ± 0.009	0.391 ± 0.009	0.397 ± 0.009	0.432 ± 0.009	<0.001
Plasma creatinine, (μmol/L)	−2.012 ± 0.736	−0.235 ± 0.718	−0.573 ± 0.719	2.555 ± 0.734	<0.001
eGFR[a] (mL/min/1.73 m^2)	−22.472 ± 0.483	−23.922 ± 0.476	−23.914 ± 0.476	−25.539 ± 0.483	<0.001

Data are shown as mean ± SD.

P-value from the general linear regression adjusted for age, sex, follow-up time and corresponding baseline value.

[a]The eGFR is based on the CKD-EPI creatinine–cystatin C equation 2012 [18] (mL/min/1.73 m^2).

AHT, anti-hypertensive treatment; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; LDL, low density lipoprotein; MDCS-CC, Malmö Diet and Cancer Study-Cardiovascular Cohort; SBP, systolic blood pressure; TG, triglycerides.

baseline levels of eGFR, SBP, BMI, fasting glucose, smoking status and use of AHT) and follow-up time to the same model additionally including p-KIM-1, we observed that including p-KIM-1 led to a marginal but significantly greater AUC (AUC 0.7872 versus $AUC_{inc.\ KIM-1}$ 0.7900; P = 0.04). Furthermore, the continuous NRI significantly improved when p-KIM-1, as a *z*-score transformed continuous variable, was added to the model, and 9.3% of the participants were reclassified into the correct risk direction (P = 0.02). Both models were adequately calibrated (Hosmer–Lemeshow's P > 0.05 for both).

Considering that further cardiovascular risk factors associated with baseline p-KIM-1 levels (Table 1), we tested if additional adjustment of the logistic regression model for waist, DBP, haemoglobin A1c (HbA1c), total cholesterol, LDL or triglycerides (TG) would affect the observed association. The results remained similar (Supplementary data, Table S2).

In an attempt to relate the impact of p-KIM-1 on the CKD outcome in relation to the other risk factors at baseline, we analysed the risk increase per increment in 1 SD change of the included variables at baseline. As expected, low eGFR was by far the strongest predictor, with a 172% [OR 2.72 (95% CI 2.39–3.11)] increased risk of future CKD per 1 SD decrease at baseline. The risk increase by 1 SD increase of p-KIM-1 at baseline was 20% [OR 1.20 (95% CI 1.08–1.33)], which was similar to the risk increase by 1 SD increase of SBP [21%; OR 1.21 (95% CI 1.09–1.35)] and comparable to that of age [55%; OR 1.55 (95% CI 1.38–1.74)] (Table 3). Similarly, the relative impact on the annual change in eGFR was strongest for baseline eGFR (0.42 mL/min/1.73 m^2 per 1 SD decrease) followed by age (−0.19 mL/min/1.73 m^2 per 1 SD increase), whereas the relative impact of p-KIM-1 was comparable to that of SBP, fasting glucose and BMI (−0.05 versus −0.06, −0.04 and −0.05 mL/min/1.73 m^2 per 1 SD increase, respectively) (Supplementary data, Table S3).

Hospitalization due to impaired renal function

During the mean follow-up time of 19.2 years (range 0–22.3), 90 participants were admitted to the hospital due to hiRF as the primary diagnosis. The sex-adjusted HR per 1 SD increase of p-KIM-1 was 1.94 (95% CI 1.63–2.30) and individuals within the highest quartile of baseline p-KIM-1 had a significantly higher risk for hiRF compared with participants within the lowest quartile [3.51 (95% CI 1.82–6.75)]. After adjusting for known risk factors (sex, baseline levels of eGFR, SBP, BMI, fasting glucose, smoking statutes and use of AHT), the HR for hiRF per 1 SD increase of p-KIM-1 remained significant [HR 1.43 (95% CI 1.18–1.74)], whereas the risk for individuals within the highest quartile of baseline p-KIM-1 was no longer significant compared with participants within the lowest quartile [HR 1.84 (95% CI 0.93–3.63)] (Table 4 and Figure 3). However, when we included 82 additional cases with hiRF as the secondary diagnosis, the risk was attenuated but remained significant [HR Q4 1.78 (95% CI 1.08–2.94)] (Table 4 and Supplementary data, Figure S1). The results remained similar when we additionally adjusted the model for further covariates associated with baseline levels of p-KIM-1 (waist, DBP, HbA1c, total cholesterol, LDL or TG and all off these together) (Supplementary data, Tables S4 and S5).

Change in eGFR among patients with diabetes at baseline

In total, 76 participants had diabetes at baseline and their mean eGFR was 93.68 mL/min/1.73 m^2 (range 57.9–138.3). Among these patients, higher baseline p-KIM-1 associated with a greater decline in eGFR during the follow-up time (P = 0.0040) (Figure 4).

DISCUSSION

In our population-based cohort of middle-aged Swedish participants, we observed an association between higher baseline fasting p-KIM-1 and greater longitudinal decline of eGFR, higher incidence of CKD and hiRF after adjusting for known risk factors for kidney dysfunction. To our knowledge, this is the first population-based study investigating the relationship between

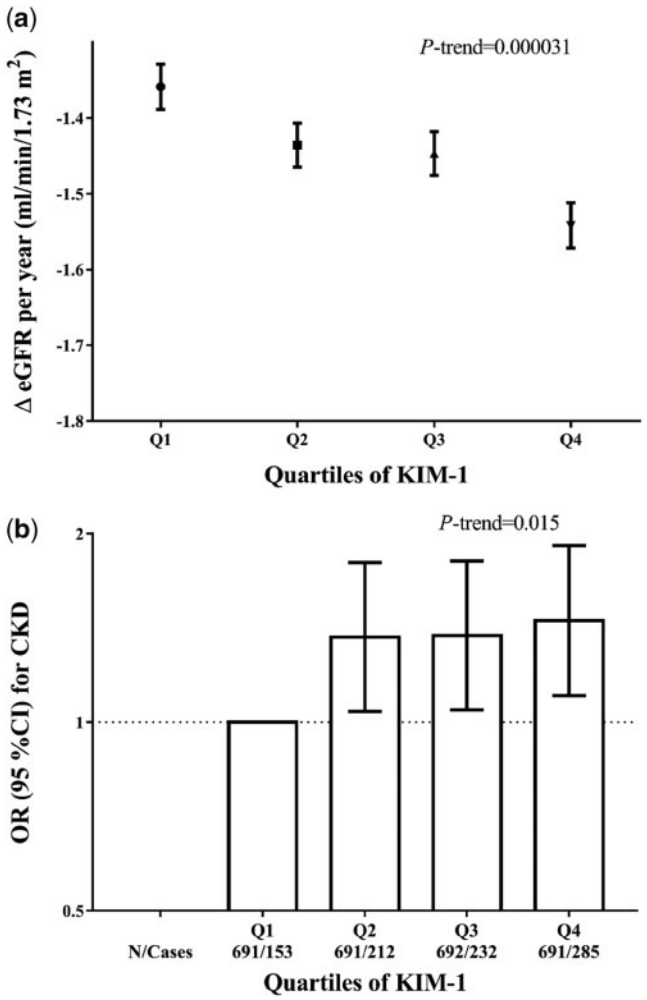

FIGURE 2: Association between baseline p-KIM-1 and decreased longitudinal kidney function and incidence of CKD in MDCS-CC. (**a**) Annual change in eGFR through quartiles of fasting p-KIM-1 at baseline among 2799 participants in the MDCS-CC during a mean follow-up time of 16.6 ±1.5 years. Data are shown as mean (SE). The GLM was adjusted for age, sex and baseline eGFR. (**b**) Incidence of CKD at follow-up examination in relation to baseline levels of p-KIM-1 among 2765 participants in the MDCS-CC. High levels of p-KIM-1 associated with an increased incidence of CKD [OR 1.45 (95% CI 1.10–1.92)]. Data are shown as OR and 95% CI from the logistic regression model adjusted for age, sex, eGFR at baseline, SBP, AHT, BMI, glucose, smoking and follow-up time. The mean follow-up time was 16.6 ±1.5 years. AHT, anti-hypertensive treatment; BMI, body mass index, eGFR, estimated glomerular filtration rate according CKD-EPI equation, [18] MDCS-CC, Malmö Diet and Cancer Study-Cardiovascular Cohort, SBP, systolic blood pressure; Q1, lowest quartile; Q4, highest quartile.

circulating p-KIM-1 and longitudinal kidney function in an apparently generally healthy population.

The gene encoding KIM-1 (*HAVCR*) is primarily expressed in the kidney cortex [22] and so far the majority of the previous human studies have investigated u-KIM-1 [3–6, 8–10]. The results of our study are in line with some previous human evidence reporting a relationship between p-KIM-1 and deterioration of kidney function in patients with T1D or early or advanced DKD [11–13], yet this study is the first to demonstrate that higher p-KIM-1 levels predict longitudinal deterioration of kidney function in a generally healthy population. Although only a limited number of individuals in our cohort had diabetes at baseline ($N = 76$), patients within the highest p-KIM-1 quartile had a significantly greater (65% increased) decline of eGFR compared with patients in the lowest quartile.

One earlier study measured both p-KIM-1 and u-KIM-1 in a cohort of T1D patients and observed that higher p-KIM-1 associated with an increased risk for early progression of renal decline independent of known clinical risk factors, including u-KIM-1, whereas this was not the case for u-KIM-1 [12]. Indeed, u-KIM-1 and p-KIM-1 are correlated ($r = 0.43$) [13], and it has been discussed that while u-KIM-1 reflects the acute production of protein that can vary widely over time, p-KIM-1 may reflect production over time and thus may be a better marker of chronic ongoing injury [12]. The two markers may also mirror diverse aspects of proximal tubular damage: u-KIM-1 reflecting the extent of damage in the proximal tubule, while the movement of KIM-1 into the circulation may be facilitated by loss of tubular cell polarity with injury, when KIM-1 may be released directly to the interstitium, or increased transepithelial permeability and disruption of the actin cytoskeletal architecture in renal microvascular endothelial cells [12, 13, 23–26].

A key factor of a biomarker is clinical applicability. When we added the p-KIM-1 baseline level to a model of commonly used risk factors, we were able to improve the classification for 9% of the participants into the correct risk direction using cNRI index analyses. However, improvement of the AUC was rather small and only marginally significant and therefore p-KIM-1 may only be moderately helpful in discrimination between individuals. Nevertheless, it needs to be taken into

Table 3. Relative impact of clinical variables at baseline on the risk increase for CKD at follow-up examination in 2765 participants in the MDCS-CC in a multivariate logistic regression analysis

Clinical characteristics	OR (95% CI)	P-value
Increase in age[a]	1.55 (1.38–1.74)	<0.001
Decrease in eGFR at baseline[a,b]	2.72 (2.39–3.11)	<0.001
Increase in FU-time[a]	1.38 (1.26–1.53)	<0.001
Increase in SBP at baseline[a]	1.21 (1.09–1.35)	<0.001
Increase in fasting glucose at baseline[a]	1.04 (0.90–1.19)	0.622
Increase in BMI at baseline[a]	1.11 (1.00–1.23)	0.052
Increase in p-KIM-1 at baseline[a]	1.20 (1.08–1.33)	0.001
Male	1.05 (0.85–1.28)	0.667
Use of AHT	1.48 (1.14–1.93)	0.003
Smoking status		
Ex	0.72 (0.56–0.93)	0.013
Current	0.80 (0.62–1.02)	0.066

[a]*z*-score transformed; the OR and 95% CI are given per 1 SD change in the respective variable.

[b]The eGFR is based on the CKD-EPI creatinine–cystatin C equation 2012 [18] (mL/min/1.73 m^2).

P-value The logistic regression model was adjusted for all variables presented in this table. All *z*-score transformed variables were entered simultaneously as continues variables and sex, use of AHT and smoking were entered as categorical variables. Participants with an eGFR <60 mL/min/1.73 m^2 at baseline were excluded prior to analysis.

AHT, anti-hypertensive treatment; BMI, body mass index; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; HDL, high density lipoprotein; LDL, low density lipoprotein; MDCS-CC, Malmö Diet and Cancer Study-Cardiovascular Cohort; SBP, systolic blood pressure; TG, triglycerides.

Table 4. Incidence of hospitalization primarily due to impairment of renal function during a mean follow-up time of 19 years in relation to baseline p-KIM-1 in the MDCS-CC

	Per quartile of p-KIM-1	Q1	Q2	Q3	Q4	Per 1 SD of log p-KIM-1
p-KIM-1[a], mean (range)						
Males		4.22 (2.22–4.66)	4.93 (4.67–5.19)	5.42 (5.19–5.66)	6.22 (5.67–10.44)	
Females		4.19 (2.34–4.63)	4.87 (4.63–5.09)	5.31 (5.09–5.56)	6.06 (5.56–8.88)	
Impairment of renal function as the primary diagnosis						
N/cases[b]	4406/90	1101/11	1102/8	1102/19	1101/52	
Cases/1000 person-years	1.06	0.5	0.4	0.9	2.6	
Sex-adjusted HR (95% CI)	1.81 (1.45–2.27)	1.00 (ref)	0.60 (0.24–1.48)	1.33 (0.63–2.81)	3.51 (1.82–6.75)	1.94 (1.63–2.30)
Risk factor[c]– adjusted HR (95% CI)	1.40 (1.11–1.76)	1.00 (ref)	0.55 (0.22–1.36)	0.99 (0.47–2.11)	1.84 (0.93–3.63)	1.43 (1.18–1.74)
Impairment of renal function, all cases						
N/cases[d]	4406/172	1101/20	1102/26	1102/36	1101/90	
Cases/1000 person-years	2.03	0.9	1.2	1.7	4.5	
Sex-adjusted HR (95% CI)	1.59 (1.36–1.86)	1.00 (ref)	1.02 (0.57–1.83)	1.33 (0.76–2.30)	3.16 (1.94–5.14)	1.77 (1.55–2.02)
Risk factor[c]– adjusted HR (95% CI)	1.27 (1.09–1.49)	1.00 (ref)	0.95 (0.53–1.71)	1.00 (0.58–1.74)	1.78 (1.08–2.94)	1.35 (1.17–1.56)

The eGFR is based on the CKD-EPI creatinine–cystatin C equation 2012 [18] (mL/min/1.73 m^2).

[a]Log-transformed.

[b]Admission to the hospital due to impairment of renal function as main diagnosis.

[c]Adjusted for sex, fasting glucose levels, eGFR, BMI, SBP, smoking status (current, former or never smokers) and use of AHT (yes/no) at baseline. Age was used as the underlying time variable.

[d]Admission to the hospital due to impairment of renal function as the main diagnosis ($n = 90$) and secondary diagnosis (additional $n = 82$; total $n = 172$).

AHT, anti-hypertensive treatment; BMI, body mass index; eGFR, estimated glomerular filtration rate; MDCS-CC, Malmö Diet and Cancer Study-Cardiovascular Cohort; SBP, systolic blood pressure.

consideration that receiver operating characteristic curve is predominantly used for diagnostic and not for prediction purposes. Moreover, both the predictive ability of the 'traditional risk model' and the strength of the new marker, as well as the potential correlation between them, impact on the effect of the change in AUC [27, 28].

In general, clinical biomarkers can be classified as either useful for prediction or as causally involved in the aetiology, thus providing valuable insights into the mechanisms of the pathophysiology. One earlier study used Mendelian randomization analysis, a genetic epidemiological approach to provide evidence for causality, and reported that among patients with T1D, genetically increased u-KIM-1 levels (normalized by urinary creatinine) were inversely associated with eGFR in cross-sectional analysis, suggesting a possible causal role for u-KIM-1 in the kidney function of T1D patients [8]. More studies on the causal role of p-KIM-1 and u-KIM-1 in renal function are warranted.

The current results show a significant but rather modest association between p-KIM-1 and kidney function in this population-based cohort. The discrimination ability of p-KIM-1 was significant, yet marginal, and showed a small improvement in the AUC when p-KIM-1 was added to a risk model. Furthermore, although cNRI was significantly improved by adding p-KIM-1, suggesting that it could be useful in reclassifying participants into correct risk directions, the relative impact (i.e. 1 SD change) of p-KIM-1 on the risk increase of CKD was rather modest compared with that of eGFR (20% versus 172% risk increase) and more comparable with that of SBP or age (21% and 55%, respectively), which may indicate a modest importance for p-KIM-1 as a clinical screening marker for risk of future CKD. However, p-KIM-1 may have clinical value regarding preventive regimes, but further studies are warranted in other large prospective population cohorts.

Our study has some limitations. One of the key limitations is the lack of data on albuminuria, which are required to determine CKD Stages 1 and 2 [29], and has in some studies shown to be of importance when investigating the association between KIM-1 levels and eGFR [8, 9]. However, in addition to studying CKD incidence, we analysed the overall change in eGFR by which we took into account the whole range of eGFRs in addition to the incidence of CKD. Second, although directly measuring GFR is hard to accomplish in a large cohort, using eGFR is a key limitation of our study. However, we used the most recent formula from the CKD-EPI, which takes into account both cystatin C as well as creatinine [18]. Third, only two measurements of creatinine and cystatin C were available in our cohort and more measurements would have been desirable according to the current Kidney Disease: Improving Global Outcomes 2012 guidelines [29]. Fourth, we used the cut-off for defining incident CKD as an eGFR of <60 mL/min/1.73 m^2 at the follow-up examination. Different views on how CKD should be classified exist, particularly concerning classification of the elderly [30], and using Stage 3A to define incident CKD could have led to an overdiagnosis, which may explain the high incidence rate of 31% in the MDCS-CC. However, we additionally investigated the relationship with hiFR. Indeed, the incidence rate of hiFR was far lower, yet the association with p-KIM-1

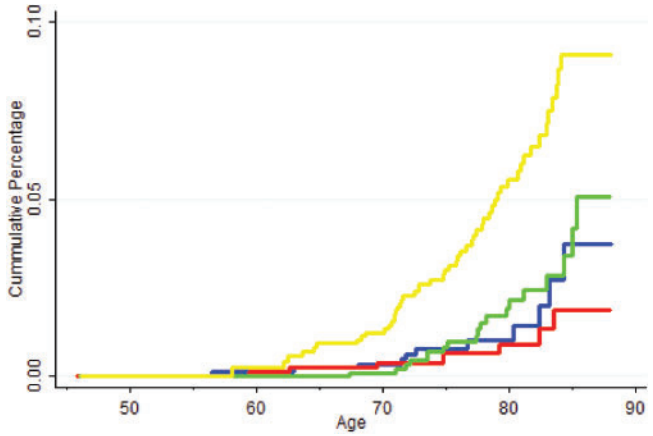

FIGURE 3: Association between baseline p-KIM-1 and hospitalization primarily due to impairment of renal function during follow-up in the MDCS-CC. Kaplan–Meier plot shows the cumulative percentage of hospitalization primarily due to impairment of renal function ($n = 90$) during a mean follow-up of 19.2 ± 4.0 years according to quartiles of baseline p-KIM-1 (Q1, lowest; Q4, highest) among 4406 participants in the MDCS-CC. Median (range) concentrations of Q1–Q4 are shown in Table 4. The Cox regression was adjusted for sex, fasting glucose levels, eGFR, BMI, SBP, smoking status (current, former or never smokers) and use of AHT (yes/no) at baseline. Age was used as the underlying time variable. In the final model, in addition to KIM-1, male sex [HR 2.62 (95% CI 1.69–4.07)], BMI [HR 1.10 (95% CI 1.05–1.15)], baseline glucose [HR 1.21 (95% CI 1.12–1.31)] and eGFR [HR 0.95 (95% CI 0.94–0.96)] were significantly associated with hospitalization primarily due to impairment of renal function. The PH assumption was fulfilled in all hiRF analyses (global P-values of 0.50 and 0.42 in primary and all hiRF analyses, respectively). AHT, anti-hypertensive treatment; BMI, body mass index; eGFR, estimated glomerular filtration rate according CKD-EPI equation [18]; MDCS-CC, Malmö Diet and Cancer Study-Cardiovascular Cohort; SBP:systolic blood pressure.

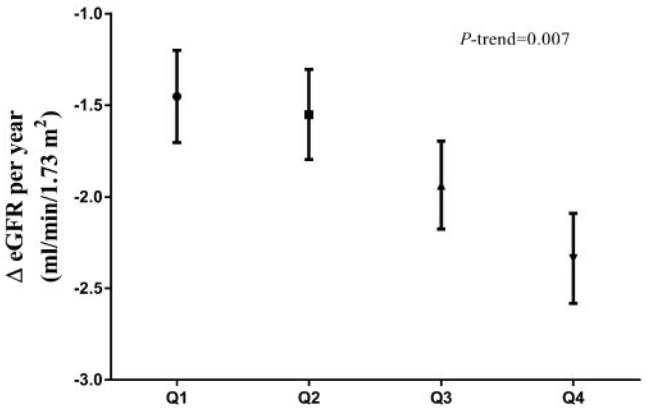

FIGURE 4: Association between p-KIM-1 and decreased longitudinal kidney function among 76 patients with diabetes at baseline in the MDCS-CC. Patients within the highest quartile of p-KIM-1 had a significantly greater decrease compared with participants in the lowest quartile [Q4: −2.34 (SE 0.25) versus Q1: −1.45 (SE 0.25) mL/min/1.73m^2; P-trend = 0.007]. Annual change in eGFR according to quartiles of baseline p-KIM-1 (Q1, lowest; Q4, highest). Data are shown as mean (SE). The GLM was adjusted for age, sex and baseline eGFR. eGFR, estimated glomerular filtration rate according CKD-EPI equation [18].

was comparably strong. Fifth, we were unable to quantify u-KIM-1. However, recent studies have reported that circulating KIM-1 correlates with u-KIM-1 ($r = 0.25$–0.43) [12, 13] and that p-KIM-1 may reflect both acute and chronic kidney injuries [13] and is associated with a rapid decline of kidney function in T1D patients, independent of u-KIM-1, whereas u-KIM-1 did not associate with renal function when adjusted for p-KIM-1 [12]. Sixth, the number of participants with diabetes at baseline was low and therefore the observed greater impact of p-KIM-1 related to eGFR decline in patients with diabetes in our study needs to be further investigated. Lastly, given the observational design of our study, we are able to show longitudinal associations but cannot prove causality.

Nevertheless, our study also has some important strengths. To our knowledge, this is the first study investigating p-KIM-1 in a prospective population-based cohort of generally healthy participants, which expands the generalizability for using p-KIM-1 as a longitudinal marker for kidney function. Furthermore, our study had a long follow-up time, convincing results were obtained utilizing several endpoints and the results remained significant when adjusted for traditional risk factors.

In conclusion, our data from a prospective population-based study in generally healthy middle-aged participants suggest that elevated p-KIM-1 associates with a longitudinal decline of eGFR, increased incidence of CKD and hiRF. Therefore our study adds to the previous evidence highlighting the importance of p-KIM-1 as a potential predictive marker of kidney dysfunction in the general middle-aged population.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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AUTHORS' CONTRIBUTIONS

G.E., J.N., O.M. and M.O.-M. contributed to the research idea and study design. G.E., P.M.N., O.M. and M.O.-M. contributed to data acquisition. C.-A.S., G.E., P.A., A.C. and M.O.-M. contributed to data analysis/interpretation. C.-A.S. and M.O-M. contributed to statistical analysis. G.E., A.C., M.O.-M. contributed to supervision or mentorship. Each author contributed important intellectual content during manuscript drafting for the overall work.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no other relevant financial interests related to this study. Preliminary results of this study were orally presented during the 52nd annual meeting of the European Association for the Study of Diabetes (EASD 2016: Oral Presentation 211).

(See related article by Arici. Kidney injury molecule-1: a successful quest for a predictive kidney disease marker? *Nephrol Dial Transplant* 2020; 35: 194–197)

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