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Cancer Epidemiology, Biomarkers & Prevention

Research Article

Serum Vascular Adhesion Protein-1 Level Predicts Risk of Incident Cancers in Subjects with Type II Diabetes

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Abstract

Background: Serum vascular adhesion protein-1 (VAP-1) predicts cancer-related mortality in diabetic subjects. However, whether serum VAP-1 predicts cancer incidence or cancer progression remains unclear. We conducted a cohort study to investigate whether serum VAP-1 and related clinical variables predict incident cancers in type II diabetic subjects.

Methods: From 1996 to 2003, we enrolled 568 type II diabetic subjects who were free of cancer at baseline. Serum VAP-1 at enrollment was measured by time-resolved immunofluorometric assay. Chronic kidney disease (CKD) was defined as estimated glomerular filtration rate <60 mL/min per 1.73 m^2. The subjects were followed until first occurrence of cancer or until December 31, 2011.

Results: During a mean follow-up of 11.3 years, 71 subjects developed incident cancers. The HRs for incident cancers in subjects with highest tertile of serum VAP-1 and in subjects with CKD were 2.95 [95% confidence interval (CI), 1.31–6.63; $P = 0.009$] and 2.29 (95% CI, 1.18–4.44; $P = 0.015$), respectively, after multivariate adjustment. There was an interaction between serum VAP-1 and CKD on the risk of incident cancers ($P = 0.01$ for log-transformed VAP-1 × CKD). The relationship among serum VAP-1, CKD, and incident cancers was similar if death was considered in the competing risk models or if subjects with shorter follow-up period were excluded.

Conclusions: Higher serum VAP-1 and CKD can independently predict future development of cancers in type II diabetic subjects.

Impact: Physicians should be aware of the early signs of cancer in diabetic individuals with elevated VAP-1 or renal dysfunction. More aggressive treatment strategies might be considered. *Cancer Epidemiol Biomarkers Prev; 23(7); 1366–73. ©2014 AACR.*

Introduction

Diabetes mellitus has emerged as a risk factor of a variety of cancers (1). The prevalence of cancers in subjects with diabetes is higher than those without diabetes (2). Therefore, it is important to identify subjects with diabetes at higher risk of cancers. In this high-risk group, regular screening program with shorter interval can detect cancers earlier, which may improve the prognosis of subjects with coexisting diabetes and cancers.

Recently, we have reported that serum vascular adhesion protein-1 (VAP-1) can predict cancer-related mortality in subjects with diabetes, above and beyond traditional risk factors (3). VAP-1 is an endothelial adhesion molecule involved in leukocyte rolling, adhesion, and transmigration into sites of inflammation (4–6). VAP-1 is also an enzyme, semicarbazide-sensitive amine oxidase (SSAO), which catalyzes oxidative deamination of primary amines into aldehydes, hydrogen peroxide, and ammonia (7). We have shown that serum VAP-1 is elevated in subjects with diabetes (8), renal dysfunction (9), and atherosclerosis (10). In addition, recent studies have implicated VAP-1/SSAO may play a crucial role in tumor progression (11, 12). Individuals with colorectal cancers showed increased serum VAP-1 when compared with healthy volunteers (13).

Although serum VAP-1 can predict cancer-related mortality in subjects with type II diabetes, whether this risk factor is correlated with cancer incidence, cancer progression, or metastasis remains unknown. The aim of this study is to investigate whether serum VAP-1 can independently predict future development of cancers in subjects with type II diabetes. Besides, we have reported

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that serum VAP-1 is associated with chronic kidney disease (CKD; ref. 9). As CKD is also an important risk factor for incident cancers (14–16), we also explored the effect of CKD on the relationship of serum VAP-1 and incident cancer in this study. In contradistinction to cause-specific analysis, we further developed a formal competing-risks (Fine-Gray) model (17) that looks at the cumulative incidence of cancer while also taking into account the informative censoring due to competing risk.

Materials and Methods

Subjects

We performed a prospective cohort study (3, 18). Between July 1996 and June 2003, subjects with type II diabetes who were regularly followed up at outpatient clinics at the Division of Endocrinology and Metabolism, National Taiwan University Hospital (Taipei, Taiwan) were invited consecutively to participate in the study. The diagnosis of type II diabetes was confirmed by endocrinologist, according to the definitions of the American Diabetes Association (19). We excluded the following patients from the analysis: (i) patients with type I diabetes or missing details on types of diabetes, (ii) patients receiving insulin treatment within 3 years after diagnosis of diabetes, (iii) patients with missing values for the determination of renal function and serum VAP-1 levels, and (iv) patients with a diagnosis of cancer at baseline. Written informed consent was obtained from each subject, and the study protocol was reviewed and approved by the Institutional Review Board.

Each subject was interviewed and underwent a physical examination by physicians. Venous blood sampling was performed after overnight fasting for the determination of plasma glucose, hemoglobin A1c (HbA1c), serum total cholesterol, triglyceride, and creatinine by using an automatic analyzer (Toshiba TBA 120FR, Toshiba Medical Systems Co., Ltd.). Serum samples were stored at −80°C in a refrigerator before the measurement of VAP-1. We also obtained information on the medications prescribed to control hypertension, diabetes, and dyslipidemia. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or medical treatment for hypertension.

Measurement of renal function

Estimated glomerular filtration rate (GFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (20). CKD was defined if estimated GFR <60 mL/min per 1.73 m^2. In addition, spot urine samples were collected to determine the presence of proteinuria by performing reflectance colorimetry (Arkray AX4280). The presence of proteinuria was defined as protein 1+ or greater.

Measurement of serum VAP-1

Serum VAP-1 and its SSAO activity are quite stable. When stored properly at −70°C, it has been shown to remain intact after 2 years (21). Serum VAP-1 was measured by time-resolved immunofluorometric assay. Briefly, the assay utilized a biotin-conjugated monoclonal anti-human VAP-1 antibody (Biotie Therapies Corp.) as a capturer on a streptavidin-coated microtiter plate. Detection of bound serum VAP-1 was performed using a different europium-conjugated anti-human VAP-1 antibody (Biotie Therapies Corp.). The time-resolved fluorescence was measured using a fluorometer (Victor^2 Multilabel Counter, PerkinElmer Finland Oy) at 615 nm. Serum VAP-1 concentration was quantified on the basis of a reference sample of highly purified human serum VAP-1 (Biovian Ltd). The R^2 of the standard curves was 0.997 to 1.000. The intra-assay coefficients of variation were 3.7%, 5.2%, and 8.9% for quality control samples with concentrations 1,000, 500, and 100 ng/mL, respectively. The inter-batch coefficients of variation from quality control samples were 4.4% to 10.2%.

Case identification and follow-up time

Patients were followed until first occurrence of cancer or December 31, 2011. Vital status, date of death, and cause of death of all subjects were obtained from the computerized death certificates maintained by the Department of Health, Executive Yuan in Taiwan. Incident cancers were ascertained by and confirmed by pathology report, medical record, and/or death certificate. The end point of this study was defined as incident cancer during the follow-up period.

Statistical analysis

Categorical variables were reported as the percentage of patients in the subgroup. The distributions of continuous variables were examined by the Shapiro–Wilk test. Continuous variables distributed normally were presented as means and SDs. Continuous variables with skewed distribution were analyzed after logarithmic transformation and were presented as medians (interquartile ranges). The Student t tests, χ^2 tests, and ANOVA were used to identify the differences in clinical characteristics between subjects with and without incident cancer, the status of CKD, and among subgroups by serum VAP-1 tertiles.

Cumulative incidence of cancer in subgroups was estimated by the Kaplan–Meier method and was tested by log-rank test. Cox proportional hazard models were applied to estimate the HRs of predictors for incident cancers. Variables significantly associated with event in univariate Cox proportional hazard models and clinically important variables were included in multivariate analyses. Differences in area under the area under the receiver-operating characteristic (ROC) curve with and without the indicated variable were calculated to determine whether the indicated variable can enhance the predictive ability for incident cancer. We have validated an area under ROC curve (AUC) results by 10-fold cross-validation for 1,000 times. Besides, we also performed competing-risk

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analyses, based on the method by Fine and Gray (17). Death was considered as the competing risk. The strength of the association between each predictor and the outcome was assessed using the subdistribution hazard ratio (SHR), which is the ratio of hazards associated with the cumulative incidence function in the presence of and in the absence of a risk factor. A two-tailed P value below 0.05 was considered significant. Stata/SE 11.0 for Windows (StataCorp LP) was used for statistical analyses.

Results

We included 568 subjects with type II diabetes (281 men and 287 women), with a mean age of 61.8 ± 9.6 years, a mean HbA1c of 7.7% ± 1.4%, and a mean estimated GFR of 75.6 ± 20.7 mL/min per 1.73 m^2. The mean duration of diabetes was 10.1 ± 8.0 years. The baseline prevalence of proteinuria, CKD, smoking, and hypertension were 17.1%, 22.0%, 17.3%, and 61.4%, respectively. During the follow-up period of 11.3 ± 3.7 years (6,429 person-years), 71 subjects had incident cancer. Hepatobiliary cancer (31%) was the most frequent diagnosis, followed by colorectal cancer (18%) and lung cancer (10%). Among the individuals who had no cancer diagnosis during follow-up, 125 died.

Baseline characteristics of study participants

Subjects with incident cancers during follow-up were older and had higher serum VAP-1 concentrations (Table 1). A higher percentage of these subjects had hypertension, CKD, and proteinuria. In Table 2, subjects with higher VAP-1 concentrations were older, had longer duration of diabetes, higher fasting plasma glucose, higher postprandial plasma glucose, higher HbA1c, higher serum creatinine, showed lower percentage to use sulfonylurea or biguanides, and more likely to use insulin. There were a higher percentage of women, subjects with proteinuria or CKD, and fewer smokers in the highest tertile of serum VAP-1. Supplementary Table S1 shows the baseline characteristics for subjects stratified by the presence of CKD. Significant differences between groups were found in age, prevalent hypertension, duration of diabetes, estimated GFR, the presence of proteinuria, current medication (e.g., biguanides, insulin, angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers), and serum VAP-1 concentrations.

Cancer risk in cause-specific analysis

The crude incidence rate of cancers per 1,000 person-years was 8.6 among subjects in the lowest tertile of serum VAP-1, 8.5 in the middle tertile and 16.7 in the highest tertile. The crude incidence rate of cancers was 8.6 events/1,000 person-years for subjects without CKD and 21.5 events/1,000 person-years for subjects with CKD. Figure 1 shows the cumulative incidence curves of cancers divided by serum VAP-1 tertiles or the presence of CKD. Subjects with serum VAP-1 in the highest tertile and subjects with CKD had a higher rate of incident cancers (both $P < 0.05$ by log-rank test).

The HRs of serum VAP-1, CKD, and proteinuria for incident cancers were calculated using the Cox proportional hazard models (Table 3). In univariate analysis, serum VAP-1, CKD, and proteinuria were associated with incident cancers. However, there was no statistically significant relationship between proteinuria and incident cancers in adjusted models. The HRs for incident cancers in the highest tertile of serum VAP-1 and the presence of CKD were 2.34 [95% confidence interval (CI), 1.11–4.91; $P = 0.025$] and 2.33 (95% CI, 1.23–4.41; $P = 0.010$), respectively, after adjusting for age, gender, smoking, fasting plasma glucose, postprandial plasma glucose, HbA1c, and proteinuria. Using log-transformed VAP-1 as a continuous variable, instead of the tertiles of VAP-1, the adjusted HR of log-transformed VAP-1 for incident cancers was 5.07 (95% CI, 1.90–16.08; $P = 0.002$). The model showed AUC of 0.71 (95% CI, 0.64–0.78). The increment in AUC was 0.04 for serum VAP-1 and 0.02 for the presence of CKD. The AUC by 10-fold cross-validation was 0.66 (95% CI, 0.25–0.97). The AUC is similar to the findings in Multi-Ethnic Cohort Study (22) which predicts incident colorectal cancer (AUC in men = 0.681 and AUC in women = 0.679). Both results suggest that further studies are needed to explore additional risk factors which can improve prediction of incident cancers. Moreover, these results were similar with further adjustment for body mass index, hypertension, duration of diabetes, total cholesterol, triglyceride, and medications at enrollment.

In addition, we also observed an interaction between serum VAP-1 and the presence of CKD on the risk of incident cancers ($P = 0.01$ for log-transformed VAP-1 × CKD). Stratified by the presence of CKD, multivariable Cox proportional hazard models were performed. In subjects without CKD, those with higher serum VAP-1 had increased risk of incident cancers (HR 10.77; 95% CI, 2.42–48.04; $P = 0.02$, adjusted for age, gender, smoking, fasting plasma glucose, postprandial plasma glucose, HbA1c, and proteinuria). However, there was no statistically significant relationship between serum VAP-1 and incident cancers in subjects with CKD (adjusted HR 1.67; 95% CI, 0.27–10.26; $P = 0.582$).

Sensitivity analysis

To minimize the influence of undiagnosed cancers at baseline, we performed sensitivity analyses (Supplementary Table S2). Exclusion of subjects with shorter follow-up period did not have major impact on the relationship between serum VAP-1, CKD, and cancer incidence. Subjects with higher serum VAP-1 and subjects with CKD were significantly associated with higher risk of incident cancers, after excluding subjects with follow-up period shorter than 1 to 4 years. A similar trend was found when excluding subjects with follow-up period shorter than 5 years, although with borderline statistical significance due to reduced

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Table 1. Baseline characteristics of the study cohort stratified according to occurrence of cancer during follow-up period

	Noncancer	Cancer	*P*
N (%)	497 (87.5)	71 (12.5)	
Age, y	61 ± 10	64 ± 8	**0.03**
Female (*N*, %)	251 (51)	36 (51)	1.0
Smoking (*N*, %)	85 (17)	13 (18)	0.8
BMI, kg/m^2	24.6 ± 3.3	24.9 ± 3.3	0.5
Hypertension (*N*, %)	297 (60)	52 (73)	**0.03**
Duration of diabetes, y	8 (3–15)	10 (5–15)	0.3
Fasting plasma glucose, mmol/L	8.4 ± 2.6	7.9 ± 2.2	0.14
Postprandial plasma glucose, mmol/L	11.9 ± 4.1	12.0 ± 4.6	0.8
HbA1c,%	7.7 ± 1.5	7.6 ± 1.3	0.7
HbA1c, mmol/mol	61 ± 16.5	60 ± 14.3	0.7
Creatinine, μmol/L	68.6 (61.0–83.9)	76.3 (61.0–99.1)	0.07
CKD (*N*, %)	99 (20)	26 (37)	**0.001**
Proteinuria (*N*, %)	79 (16)	18 (25)	**0.048**
Total cholesterol, mmol/L	5.2 ± 1.0	5.3 ± 1.2	0.9
Triglycerides, mmol/L	1.5 (1.1–2.2)	1.6 (1.2–2.2)	0.2
Medications at enrollment			
Sulfonylureas (*N*, %)	325 (65)	47 (66)	0.9
Biguanides (*N*, %)	264 (53)	31 (44)	0.14
Thiazolidinediones (*N*, %)	9 (2)	1 (1)	0.8
Insulin (*N*, %)	125 (25)	23 (32)	0.2
ACEIs or ARBs (*N*, %)	89 (18)	18 (25)	0.13
Statins (*N*, %)	23 (5)	1 (1)	0.2
Serum VAP-1, ng/mL	689 (581–819)	749 (601–968)	**0.002**
By tertile			**0.041**
Middle, 622–780 (%)	171 (34)	19 (27)	
Highest, ≥780 (%)	156 (31)	33 (46)	

NOTE: Mean ± SD or medians (interquartile ranges) are shown. Bold values signify statistically significant estimates ($P < 0.05$).
Abbreviations: BMI, body mass index; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers.

sample size ($P = 0.062$ for serum VAP-1 and $P = 0.054$ for CKD).

Competing risk analysis

After accounting for the competing risk of death due to other causes, patients in the highest tertile of serum VAP-1 had higher risk of incident cancers compared with those in the lowest tertile of serum VAP-1, adjusting for age, gender, smoking, fasting plasma glucose, postprandial plasma glucose, HbA1c, body mass index, hypertension, duration of diabetes, total cholesterol, triglyceride, and medications at enrollment (SHR 2.24; 95% CI, 1.01–4.95; $P = 0.047$). The presence of CKD also showed a trend of increased cancer risk in adjusted models (SHR 2.05; 95% CI, 0.99–4.25; $P = 0.052$). Supplementary Fig. S1 shows the cumulative incidence function of cancers after consideration of competing risk and adjustment for age, gender, smoking, fasting plasma glucose, postprandial plasma glucose, HbA1c, body mass index, hypertension, duration of diabetes, total cholesterol, triglyceride, and medications at enrollment.

Discussion

In the present study, we have demonstrated that serum VAP-1 and CKD, but not proteinuria, can predict the risk of incident cancers independently in subjects with type II diabetes. Serum VAP-1 and CKD can also improve predictive ability for incident cancers. The findings remained similar when excluding subjects whose cancers developed 1 to 4 years during follow-up and when death was considered as a competing event.

To our best knowledge, this is the first study to investigate serum VAP-1 as a risk factor for future development of cancers in patients with type II diabetes. People with diabetes are at significantly higher risk for many forms of cancers (1). Although type II diabetes and cancers share many risk factors, potential biologic links between the two diseases remain unclear. Since serum VAP-1 was higher in subjects with diabetes (8), VAP-1/SSAO may serve a link between diabetes and cancer. Indeed, subjects with colorectal cancer had higher serum VAP-1 than healthy volunteers (13). In subjects with lung cancer, serum SSAO activity has been

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Table 2. Baseline characteristics stratified by serum VAP-1 tertile in people with type II diabetes

Serum VAP-1 tertile (ng/mL)	<622	622–780	≥780	*P*
N	189	190	189	
Incident cancer (*N*, %)	19 (10)	19 (10)	33 (17)[a,b]	**0.041**
Age, y	60 ± 9	62 ± 10[a]	64 ± 9[a,b]	**0.0001**
Female (*N*, %)	82 (43)	96 (51)	109 (58)[a]	**0.02**
Smoking (*N*, %)	41 (22)	34 (18)	23 (12)[a]	**0.048**
BMI, kg/m^2	24.8 ± 3.0	24.8 ± 3.2	24.4 ± 3.7	0.4
Hypertension (*N*, %)	114 (60)	119 (63)	116 (61)	0.9
Duration of diabetes, y	5 (2–12)	8 (4–13)[a]	11 (6–18)[a,b]	**<0.0001**
Fasting plasma glucose, mmol/L	7.7 ± 1.9	8.3 ± 2.3[a]	9.0 ± 2.9[a,b]	**<0.0001**
Postprandial plasma glucose, mmol/L	11.2 ± 3.6	11.5 ± 3.5	13.0 ± 4.8[a,b]	**0.0008**
HbA1c,%	7.1 ± 1.3	7.7 ± 1.3[a]	8.2 ± 1.6[a,b]	**<0.0001**
HbA1c, mmol/mol	54 ± 14.3	61 ± 14.3[a]	66 ± 17.6[a,b]	**<0.0001**
Creatinine, μmol/L	68.6 (61.0–83.9)	68.6 (61.0–83.9)	68.6 (61.0–91.5)[a,b]	**0.016**
Proteinuria (*N*, %)	20 (11)	22 (12)	55 (29)[a,b]	**<0.0001**
CKD (*N*, %)	27 (14)	35 (18)	63 (33)[a,b]	**<0.0001**
Total cholesterol, mmol/L	5.1 ± 0.9	5.4 ± 1.0[a]	5.3 ± 1.1[a]	**0.007**
Triglycerides, mmol/L	1.5 (1.2–2.3)	1.7 (1.2–2.2)	1.4 (1.0–2.1)	0.16
Medication at enrollment				
Sulfonylureas (*N*, %)	133 (70)	132 (69)	107 (57)[a,b]	**0.007**
Biguanides (*N*, %)	105 (56)	108 (57)	82 (43)[a,b]	**0.015**
Thiazolidinediones (*N*, %)	1 (1)	4 (2)	5 (3)	0.3
Insulin (*N*, %)	27 (14)	36 (19)	85 (45)[a,b]	**<0.0001**
ACEIs or ARBs (*N*, %)	32 (17)	39 (21)	36 (19)	0.7
Statins (*N*, %)	4 (2)	13 (7)[a]	7 (4)	0.067

NOTE: Mean ± SD or medians (interquartile ranges) are shown. Bold values signify statistically significant estimates ($P < 0.05$).
Abbreviations: BMI, body mass index; ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin II receptor blockers.
[a]$P < 0.05$ versus first tertile (serum VAP-1 <622 ng/mL).
[b]$P < 0.05$ vs. second tertile (serum VAP-1 622–780 ng/mL).

shown to be associated with VEGF (11). Besides, subjects of prostate cancer with bone metastasis had higher serum SSAO activity than those without metastasis (23). In 2011, we found that patients with diabetes with higher serum VAP-1 had increased risk for cancer-related mortality (3). In this present study, we showed that increased risk of cancer incidence is one of the reasons for the increased mortality. Further studies should be done to investigate if serum VAP-1 is associated with cancer progression and metastasis in patients with diabetes.

Several potential mechanisms linking VAP-1 and incident cancers have been proposed. VAP-1 can enhance leukocyte trafficking and promote inflammatory process

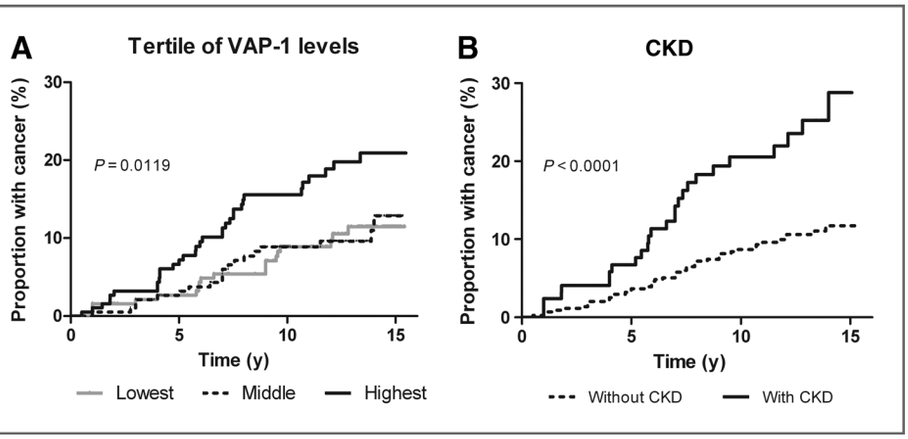

Figure 1. Kaplan–Meier curves for cumulative incidence of cancer among subgroups (A) stratified by tertile of serum VAP-1. Solid line, highest tertile; dashed line, middle tertile; gray line, lowest tertile. B, stratified by the presence of CKD. Solid line, subjects with CKD; dashed line, subjects without CKD.

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Table 3. HRs and 95% CIs for the risk of incident cancers in people with type II diabetes

	HR (95% CI)	*P*
Unadjusted analyses		
Log-transformed VAP-1 (ng/mL)	5.64 (2.20–14.51)	**<0.0001**
VAP-1 tertile		
Lowest, <622 ng/mL	1	
Middle, 622–780 ng/mL	1.00 (0.53–1.89)	1.0
Highest, ≥780 ng/mL	1.97 (1.12–3.47)	**0.018**
CKD	2.54 (1.56–4.11)	**<0.0001**
Proteinuria	2.20 (1.26–3.69)	**0.005**
Adjusted analyses[a]		
Model 1[b]		
Log-transformed VAP-1 (ng/mL)	5.07 (1.90–16.08)	**0.002**
CKD	2.34 (1.24–4.44)	**0.009**
Proteinuria	1.53 (0.78–3.02)	0.220
Model 2[c]		
Log-transformed VAP-1 (ng/mL)	6.79 (2.03–22.67)	**0.002**
CKD	2.31 (1.19–4.48)	**0.013**
Proteinuria	1.51 (0.74–3.08)	0.3
Model 3[b]		
VAP-1 tertile		
Lowest, <622 ng/mL	1	
Middle, 622–780 ng/mL	1.15 (0.53–2.48)	0.732
Highest, ≥780 ng/mL	2.34 (1.11–4.91)	**0.025**
CKD	2.33 (1.23–4.41)	**0.010**
Proteinuria	1.59 (0.81–3.12)	0.173
Model 4[c]		
VAP-1 tertile		
Lowest, <622 ng/mL	1	
Middle, 622–780 ng/mL	1.21 (0.53–2.76)	0.7
Highest, ≥780 ng/mL	2.95 (1.31–6.63)	**0.009**
CKD	2.29 (1.18–4.44)	**0.015**
Proteinuria	1.53 (0.76–3.08)	0.2

NOTE: Bold values signify statistically significant estimates ($P < 0.05$).

[a]Serum VAP-1, CKD, and proteinuria were all included in adjusted models.

[b]Further adjusted for age, gender, smoking, fasting plasma glucose, postprandial plasma glucose, and HbA1c.

[c]Further adjusted for age, gender, smoking, fasting plasma glucose, postprandial plasma glucose, HbA1c, body mass index, hypertension, duration of diabetes, total cholesterol, triglyceride, and medications at enrollment (e.g., sulfonylureas, biguanides, thiazolidinediones, insulin, angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, statins).

(4, 24). Inflammation has been hypothesized to increase the risk for cancers (25). In addition, the SSAO activity of VAP-1 can catalyze oxidative deamination reaction to produce hydrogen peroxide, a source of oxidative stress, and aldehyde, a precursor of advanced glycated end products (AGE; ref. 7). Enhanced oxidative stress (26) and the interaction between AGEs and its receptor, receptor of AGEs (RAGE; ref. 27), were both associated with the development of cancers. RAGE has been identified in both epithelial and mesenchymal cells and is upregulated in intestinal adenomas (27). In RAGE-knockout mice, tumor cells showed increased apoptosis and intestinal tumorigenesis was reduced (28). All these findings support our observation that serum VAP-1 is associated with incident cancers in the present study.

Interestingly, our findings are consistent with the previous literature, showing a close relationship between CKD and cancers. Many reports have demonstrated that patients on renal replacement therapy for end-stage renal disease, either dialysis or transplantation, are at higher risk for cancers (15, 29–31). This increased risk was also observed in subjects with mild to moderate kidney disease. Wong and colleagues (14) showed that men but not women with CKD stage 3 or more had a significantly increased risk for cancers. In addition, there have been studies showing an increased risk of cancer-related mortality in elderly subjects with CKD (32). A large cohort study in the general population in Taiwan found that patients with CKD had a higher risk for overall cancer morality (33). Because

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diabetes and CKD are both independently risk factors of cancer, it is reasonable to hypothesize that diabetes complicated by CKD would further augment risk of cancer. In subjects with type II diabetes, the present study is the first one to show that CKD is associated with increased risk of cancer development. In contrast, a *post hoc* analysis of the ADVANCE study showed mild to moderate CKD does not increase the risk of incident cancers in subjects with type II diabetes (34). As the follow-up period in the ADVANCE study is shorter than that in our study (5 vs. 11 years), this could be one of the reasons for the different results.

The underlying mechanisms for the association between CKD and cancer remain unclear. Here are some hypotheses. First, the prevalence of vitamin D deficiency is high among patients with moderately reduced kidney function (35). Because there is emerging evidence supporting the association between vitamin D deficiency and increased risk of certain cancers (36–38), vitamin D deficiency may be one of the potential mechanisms for the association between CKD and increased risk of cancers. Second, CKD is a reflection of inflammatory process (39), and chronic inflammatory process has been associated with increase the risk of various cancers (25).

However, the somewhat surprising finding of the present study was a lack of association between proteinuria and incident cancers. Our findings were supported by another study. They also failed to show significant relationship between proteinuria and cancer risk in subjects with diabetes, although the copresence of proteinuria and low-density lipoprotein cholesterol <2.80 mmol/L were associated with increased cancer risk (40). In our previous report, proteinuria can predict cancer-related mortality in patients with type II diabetes (18). Taken together, these findings suggest that proteinuria might be a marker of cancer progression or metastasis in subjects with diabetes. Besides, the relationship between proteinuria and cancers may be different between subjects with diabetes and without diabetes. The Tromsø study found that albuminuria correlated with the incident cancers significantly in individuals without diabetes (16). The interaction and relationship among proteinuria, cancer progression, and diabetes should be investigated in further studies.

The strength of this study is its long follow-up period, with an average of 11.3 years. The highly sensitive time-resolved immunofluorometric assay for measuring serum VAP-1 enabled us to differentiate subtle differences in circulating VAP-1 concentrations. Besides, the present study adjusted most, if not all, important confounders in statistic models, performed sensitivity tests, and analyzed the data in competing-risk models in additional to Cox proportional hazard models. However, our study had some limitations. First, the time-dependent changes of VAP-1 during the follow-up period were not assessed. Second, the present study only enrolls Han Chinese, which limits the generalization of the findings.

In conclusion, we have demonstrated that serum VAP-1 and CKD can predict future development of cancers in subjects with type II diabetes independently. Further studies are needed to investigate the detailed mechanisms, the use of these markers for risk stratification, and the potential applications of the findings to guide screening and treatment strategies in subjects with diabetes.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): H.-Y. Li, Y.-D. Jiang, T.-J. Chang, L.-M. Chuang
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): T.-Y. Yu, H.-Y. Li, J.-N. Wei, L.-M. Chuang
Writing, review, and/or revision of the manuscript: T.-Y. Yu, H.-Y. Li, L.-M. Chuang
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): H.-Y. Li, T.-J. Chang, C.-M. Lin, C.-C. Chu, L.-M. Chuang
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