学位論文

High FIB4 index is an independent risk factor of diabetic kidney disease in type 2 diabetes

（FIB4 index は 2 型糖尿病患者における糖尿病性腎臓病の独立した予測因子である）

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論文内容要旨

学位論文題名	High FIB4 index is an independent risk factor of diabetic kidney disease in type 2 diabetes （FIB4 index は 2 型糖尿病患者における糖尿病性腎臓病の独立した予測因子である）

【序文】非アルコール性脂肪性肝疾患（nonalcoholic fatty liver disease：NAFLD）や非アルコール性脂肪性肝炎（nonalcoholic steatohepatitis：NASH）と慢性腎臓病（chronic kidney disease：CKD）発症の関連が報告されている。肝生検は肝線維化を病理学的に評価できる点で有用だが、侵襲性やサンプリングエラー等の限界があり、非侵襲的スコア FIB4 index （年齢 [年] x AST [IU/L] / √ALT [IU/L] x 血小板 [10^9/L]）がしばしば代用される。FIB4 index は非糖尿病患者の CKD 発症予測能を有するとされるが、糖尿病性腎臓病での意義は不明である。本研究は FIB4 index と糖尿病性腎臓病（diabetic kidney disease : DKD）発症リスクの関係を後ろ向きコホート研究で検討した。【方法】対象は福島県立医科大学糖尿病内分泌代謝内科外来または豊見城中央病院糖尿病・生活習慣病センター外来を受診した 2 型糖尿病患者。各施設初診時に eGFR≧60 mL/min/1.73 m^2かつ試験紙法 1+以上の尿蛋白がなく、NAFLD、NASH 以外の肝疾患、原発性あるいは続発性腎疾患、血液疾患の無い症例を登録した。通院期間中の eGFR＜60 への低下または尿蛋白 1+以上の出現を DKD 発症とした。登録症例を初診時の FIB4 index＞1.3（肝線維化予測のカットオフ値）と FIB4 index≦1.3 の 2 群に分けて追跡した。【結果】対象の 2 型糖尿病患者 584 名の平均年齢は 55 ± 11 歳、男性 61.6%（360 名）、観察期間中央値 6.0（3.8～11.0）年、FIB4 index＞1.3 群（187 名、32.0%）であった。Kaplan-Meier 法（Log-rank 検定）で、FIB4 index＞1.3 群は FIB4 index≦1.3 群に比べ、DKD 発症（ハザード比 HR 1.68 [95%CI :1.32-2.14]、p<0.001）、eGFR＜60（HR1.83 [1.37-2.47]、P<0.001）、尿蛋白出現（HR1.39 [1.02-1.88]、P=0.033）いずれのハザード比も高かった。Cox 回帰分析で FIB4 index＞1.3 は、DKD 発症（HR 1.54 [1.15-2.08]、p=0.004）と蛋白尿出現（HR 1.55 [1.08-2.23]、p=0.020）の予測因子であったが、eGFR＜60（HR 1.14 [0.79-1.64]、p=0.437）の予測因子でなかった。【考察】FIB4 index＞1.3 が糖尿病性腎臓病の発症予測因子となることが示された。肝線維化に伴う DKD の発症には、1）動脈硬化の進展、2）肝臓由来の炎症性メディエーター、3）肝腎症候群、4）インスリン抵抗性など、複数の機序が推定される。研究の限界として肝線維化を病理学的に評価していない点、後ろ向き研究のため因果関係を示せない点等がある。【結論】本研究は肝線維化指標 FIB4 index＞1.3 が、2 型糖尿病患者の DKD 発症と相関することを初めて示した。この結果は FIB4 index 高値の 2 型糖尿病患者における腎機能モニタリングが重要であること、肝線維化と DKD 発症の因果関係について検証が必要であることを示唆する。

Abbreviation

BMI: body mass index

CKD: chronic kidney disease

DKD: diabetic kidney disease

ESRD: end stage renal disease

NAFL: nonalcoholic fatty liver

NAFLD: nonalcoholic fatty liver disease

NASH: nonalcoholic steatohepatitis

LC: liver cirrhosis

HbA1c: hemoglobin A1c

IR: insulin resistance

eGFR: estimated glomerular filtration rate

AST: aspartate aminotransferase

ALT: alanine aminotransferase

γGT: γ-glutamyltransferase

TC: total cholesterol

TG: triglyceride

LDL: low density lipoprotein

ACEi: angiotensin converting enzyme inhibitor

ARB: angiotensin II receptor blocker

HRS: hepatorenal syndrome

ROC: receiver operating characteristic

AUC: area under the curve

HR: hazard ratio

1. Introduction

Chronic kidney disease (CKD) associated with diabetes mellitus, often referred as diabetic kidney disease (DKD) [1-4], is the leading cause of end-stage kidney disease (ESKD) for patients with diabetes [5].The treatment of earlier stages of DKD is effective in slowing the progression toward ESRD [1-3]. Thus, early detection of precursors and/or risk factors for DKD is crucial [1-3]. Family history of DKD, smoking history, and control of glycemic, blood pressure, and plasma lipid levels are established factors for identifying people at a greater risk of DKD development and progression. Among emerging risk markers for CKD [1-4], nonalcoholic fatty liver disease (NAFLD) is also an exacerbation factor for the development and progression of CKD in the non-diabetic [6-8] and diabetic populations [9-13].

NAFLD moves pathologically from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) and cirrhosis (LC) [14]. A liver biopsy is useful to detect progressive NASH in NAFLD patients for estimation of their prognosis. However, liver biopsy has limitations such as invasiveness, sampling errors, and cost. For this reason, multiple scoring systems that noninvasively predict the progression to NASH and liver fibrosis have been proposed [15-18]. The FIB4 index is a high ability non-invasive scoring system used to predict NASH and liver fibrosis [19-21]. A relationship between the FIB4 index and onset of CKD was reported in non-diabetic patients [22], but the relationship has never been studied in a diabetic population.

Therefore, we evaluated the prognostic impact of the FIB4 index on the risk of developing DKD in Japanese type 2 diabetic patients in a single-center retrospective cohort study.

2. Materials and methods

2.1. Study design and subjects

This is an observational retrospective cohort study. The study protocol was approved by the Fukushima Medical University Ethics Committee (Number 29118) and the Tomishiro Central Hospital Ethics Committee (R01R027). This study was conducted according also to the Ethical Guidelines for Medical and Health Research Involving Human Subjects enacted by MHLW of Japan [http://www.mhlw.go.jp/file/06-Seisakujouhou-10600000-Daijinkanboukouseikagakuka/0000069410.pdf and http://www.mhlw.go.jp/file/06-Seisakujouhou-10600000-Daijinkanboukouseikagakuka/0000080278.pdf]. Inclusion criteria of the participants was adult patients with type 2 diabetes mellitus who had visited the Department of Diabetes, Endocrinology, and Metabolism, Fukushima Medical University Hospital or Department of Diabetes and Lifestyle-Related Disease Center, Tomishiro Central Hospital between January 2002 and March 2019. Written informed consent was obtained from the patients between January 2018 and March 2019 in the Department of Diabetes, Endocrinology, and Metabolism, School of Medicine, Fukushima Medical University Hospital and informed consent for participants in Tomishiro Central Hospital was waived by the Tomishiro Central Hospital Ethics Committee. Instead, we publicized information concerning this study in the Hospital and ensured that the subjects could refuse the use of their personal information. Total

of 1,197 patients with type 2 diabetes mellitus were selected from both hospitals on their medical records (Figure 1). On the below definition of DKD, 279 CKD/DKD at baseline were excluded. On exclusion criteria, 81 were removed by complications of liver, kidney and hematologic diseases (Figure 1). Twenty-four patients with non-diabetic kidney diseases (chronic glomerulonephritis, vasculitis, polycystic kidney disease, and renal cancer) and 47 patients with liver disease other than NAFLD (viral hepatitis, autoimmune liver disease, liver transplantation). Patients diagnosed with liver cirrhosis and heavy drinker (consumption of ethanol less than 20 g/day for women and 30 g/day for men) had been excluded in advance. After deleted for 146 with observation period <1 year and 107 with missing data, the remaining 584 patients with type 2 diabetes mellitus were enrolled and their paper and/or electrical medical records were scrutinized from October 2002 to March 2019. Their first visit to either hospitals was considered as the baseline. The parameters such as age, sex, history of diabetes, family and social history, medical checkup history, complications, medications, laboratory data, and all dates were recorded.

2.2. Biochemical measurements

Laboratory parameters were measured by standard assays. In brief, HbA1c was measured by ion exchange high performance liquid chromatography in automated glycohemoglobin analyzer (HLC-723G8/G9, Tosoh Corp., Tokyo, Japan). Creatinine was measured by an enzymatic assay in clinical chemistry analyzer (ARCHITECT c16000, Abbott, Illinois, USA). Semiqualitative proteinuria was assessed by urinary dipstick test.

Ultrasonography

Standard abdominal ultrasonography was performed in a part of patients after overnight fasting. Diagnosis of NAFLD was based on the increased echogenicity of the liver parenchyma as compared to the right kidney's cortex. Visibility and sharpness of the diaphragm and hepatic veins' interface were analyzed as well. Based on these 3 parameters was further classified into 3 grades: Grade 0, no steatosis; Grade 1, mild steatosis; Grade 2, moderate steatosis; Grade 3, severe steatosis as described [47].

Definition

Diabetes mellitus was defined by a fasting plasma glucose ≥126 mg/dL, random plasma glucose ≥200 mg/dL, and/or HbA1c ≥6.5% (48 mmol/mol) or use of anti-diabetic medication (World Health Organization (2006) Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation. https://www.who.int/diabetes/publications/diagnosis_diabetes2006/en/). Patients with type 1 diabetes mellitus or secondary diabetes were excluded. The FIB4 index was calculated by Age (year) × AST (IU/L) / (√ALT (IU/L) × Platelet count (10^9/L)) [29,48]. A cut-off value of 1.3 or less, which was 90% negative for the progression of liver fibrosis, was applied [16]. The definition of DKD was eGFR <60 mL/min/1.73 m^2 and/or proteinuria 1+ with urinary dipsctick test. The primary endpoint of this study was onset of DKD. The secondary endpoint of this study was

each onset of eGFR <60 mL/min/1.73 m^2 or proteinuria 1+ with a dipstick urine test. We calculated eGFR using the Japanese formula for GFR estimation, i.e., eGFR (mL/min/1.73 m^2) = 194 x serum creatinine (mg/dL) $^{-1.094}$ x age (years) $^{-0.287}$ [49]. Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or those taking antihypertensive drugs. Dyslipidemia was defined as LDL cholesterol ≥140 mg/dL or those taking antihyperlipidemic drugs.

2.3. Statistical analysis

Continuous and parametric values are expressed as mean ± standard deviation, and the nonparametric variables as median (first quartile-third quartile). The two-tailed unpaired student's t-test and Mann-Whitney U test were used for parametric and non-parametric data, respectively. Categorical variables are shown as percentages and were analyzed using the Chi-square test. Univariate survival analysis was calculated using the Kaplan-Meier curve and analyzed by a log rank test. Univariate and Cox proportional hazards model were used to determine the independent contributions of the FIB4 index as a dichotomizing variable (> 1.3 vs. ≤ 1.3) to the development of DKD, a decline in eGFR (<60 mL/min/1.73 m^2), or proteinuria after adjusting for age, sex, BMI, baseline HbA1c, baseline eGFR, smoking and drinking status (current or past), comorbidities (hypertension, dyslipidemia) and anti-diabetic and anti-hypertensive medications. Covariates used for the Cox proportional hazards model were chosen from possible confounding factors for DKD[1-4].

For sensitivity analyses: univariate and Cox proportional hazards models were repeated: 1) by using the FIB4 index as continuous or quartile variable≈; 2) by HbA1c as a time dependent cobariate plus possible emerging biomarker for DKD (white blood cell count); 3) by a new data-set with multiple imputation method for missing data analysis: 4) time dependent AUC of FIB4 Index for the develeopme of DKD, eGFR < 60 and proteinuria.

Values of P <0.05 were considered as statistically significant. Statistical analyses were conducted using SPSS version 25 (SPSS, Inc., Chicago, Illinois, USA) or R 3.6.3. VIM package 5.1.1 and ggplot2 3.3 running on R 3.6.3 are used for visualization of the missing pattern.

3. Results

3.1. General characteristics

Flow chart for study recruitment was shown in **Figure 1**. Baseline characteristics of the patients are shown in **Table 1**. Among the 584 participants with type 2 diabetes mellitus, 187 (32.0%) were FIB4 index >1.3 and 397 (68.0%) were FIB4 index ≤1.3. FIB4 index > 1.3 had a higher age (62.0 ± 8.0 vs 52.2 ± 10.0, *P* <0.001), a larger proportion of men (130 (69.1%) vs 230 (58.1%), *P* = 0.014), and a lower BMI (25.1 ± 3.8 vs 26.4 ± 5.7, *P* = 0.005). Past drinkers were higher in FIB4 index >1.3, but past smokers were similar. The prevalence of dyslipidemia was lower in FIB4 index >1.3, but that of hypertension was similar. Blood biochemistry showed that FIB4 index >1.3 had significantly fewer white blood cells and platelets and lower eGFR. There was no significant difference in HbA1c, ALT and albumin. By contrast, AST and γGT

were high and TC, TG and LDL-cholesterol were low in FIB4 index >1.3. The decrease of eGFR <60 mL/min/1.73 m^2 [41.8 % vs 26.8 %, *P* = 0.001], and onset of DKD [58.5% vs 43.7%, *P* = 0.001] were higher in FIB4 index >1.3 and that of proteinuria showed a non-significant difference (proteinuria 1+ (35.1% vs 28.3%, *P* = 0.09)

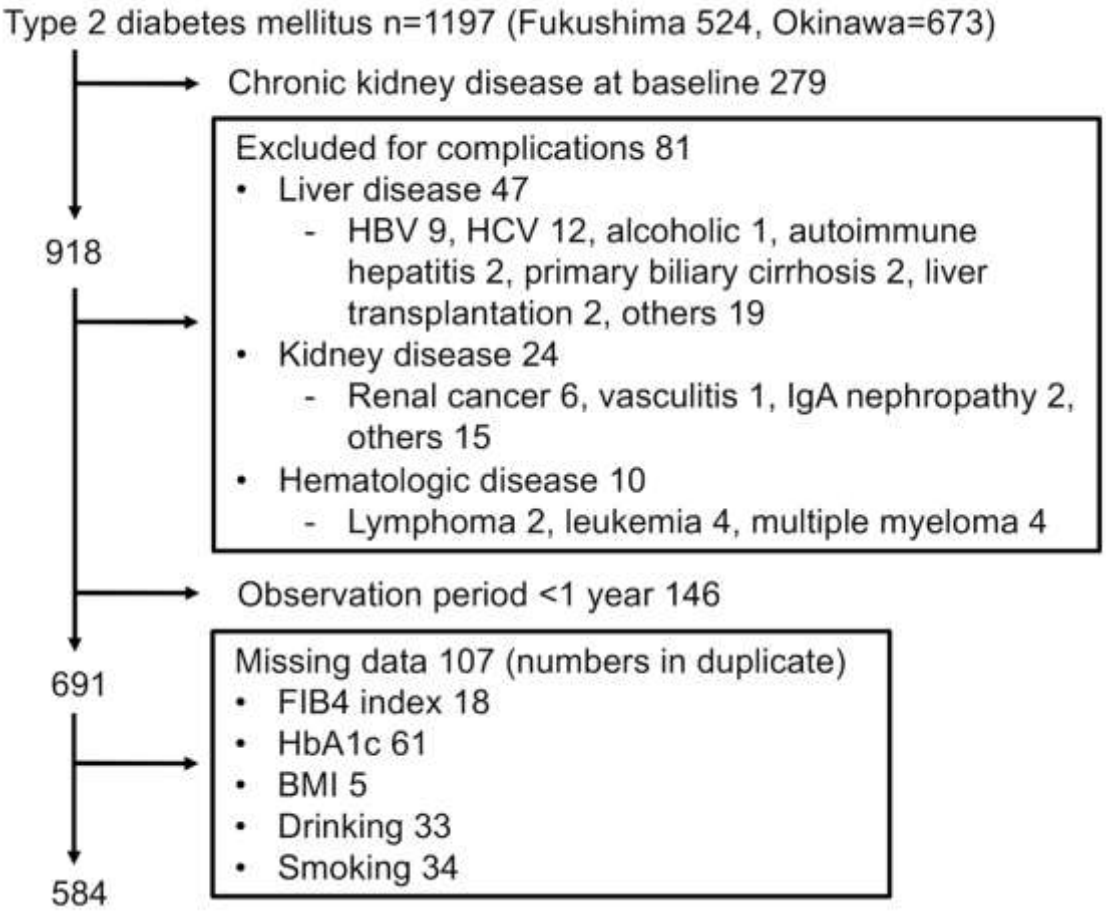


Figure 1. Flow chart for study recruitment. Total of 1,197 patients with type 2 diabetes mellitus were selected from two Japanese centers on medical records. HBV, hepatitis B virus hepatitis; HCV, hepatitis C virus hepatitis. Patients diagnosed with liver cirrhosis and heavy drinker (consumption of ethanol less than 20 g/day for women and 30 g/day for men) had been excluded in advance.

Table 1. Baseline characteristics of studied patients

Parameters	All (n=584)	FIB4 ≤ 1.3 (n=397)	FIB4 > 1.3 (n=187)	*P* value
Age, years	55.4 ± 10.5	52.2 ± 10.0	62.0 ± 8.0	<0.001
Men, n (%)	360 (61.6)	230 (58.1)	130 (69.1)	0.014
Duration of diabetes, years	14.0 (8.0-20.0)	13.0 (8.0-19.0)	14.0 (8.8-22.3)	0.141
Height, cm	160.6 ± 8.6	160.8 ± 8.8	160.0 ± 8.0	0.270
Body weight, kg	66.9 ± 13.5	67.9 ± 13.8	64.7 ± 12.4	0.005
Body mass index, kg/m^2	26.0 ± 5.2	26.4 ± 5.7	25.1 ± 3.8	0.008
Systolic blood pressure, mmHg	135 ± 19	135 ± 19	137 ± 18	0.055
Diastolic blood pressure, mmHg	82 ± 12	83 ± 12	81 ± 11	0.235
Past smoker, n (%)	286 (49.0)	197 (49.7)	89 (47.3)	0.590
Past drinker, n (%)	307 (52.6)	192 (48.5)	115 (61.2)	0.005
Dyslipidemia, n (%)	426 (72.9)	302 (76.3)	124 (66.0)	0.009
Hypertension, n (%)	359 (61.5)	233 (58.8)	126 (67.0)	0.060
Drugs				
Sulfonylurea, n (%)	92 (15.8)	56 (14.1)	36 (19.3)	0.110
Biguanide, n (%)	77 (13.2)	57 (14.4)	20 (10.7)	0.220
DPP4 inhibitor, n (%)	31 (5.3)	15 (3.8)	16 (8.6)	0.028
Thiazolidine, n (%)	27 (4.6)	18 (4.5)	9 (4.8)	0.880
αGI, n (%)	59 (10.1)	38 (9.6)	21 (11.2)	0.540
Glinide, n (%)	12 (2.1)	8 (2.0)	4 (0.7)	0.920
Insulin, n (%)	36 (6.2)	22 (5.5)	14 (7.5)	0.360
ACEi/ARB, n (%)	113 (19.4)	74 (18.7)	39 (20.9)	0.540
White blood cell, /μL	5900 ± 2600	6200 ± 2800	5200 ± 2200	<0.001
Hemoglobin, g/dL	14.1 ± 1.5	14.0 ± 1.5	14.2 ± 1.5	0.100
Platelet, 10^4/μL	23.2 ± 6.7	25.7 ± 6.4	18.1 ± 4.0	<0.001
Blood urea nitrogen, mg/dL	13.9 ± 3.9	13.7 ± 4.1	14.4 ± 3.5	0.042
Creatinine, mg/dL	0.70 ± 0.16	0.69 ± 0.16	0.72 ± 0.15	0.070
eGFR, mL/min/1.73m^2	81.9 ± 21.3	85.9 ± 18.8	79.6 ± 15.8	<0.001
Hemoglobin A1c, %	7.7 ± 2.1	7.7 ± 2.1	7.6 ± 1.9	0.340
Aspartate aminotransferase (AST), U/L	21 (17-29)	20 (16-26)	25 (20-39)	<0.001
Alanine aminotransferase (ALT), U/L	27 (19-41)	26 (19-39)	28 (20-45)	0.300
γ-glutamyl transferase (γGT), U/L	34 (21-60)	32 (21-57)	40 (22-72)	0.011
Serum albumin, g/dL	4.3 ± 0.5	4.4 ± 0.5	4.3 ± 0.4	0.410
Uric acid, mg/dL	5.3 ± 1.5	5.3 ± 1.5	5.3 ± 1.4	0.842
Total-cholesterol, mg/dL	203 ± 40	206 ± 40	197 ± 39	0.005
Triglyceride, mg/dL	130 (92-180)	139 (96-188)	117 (88-163)	0.005
HDL-cholesterol, mg/dL	51 ± 13	50 ± 13	53 ± 14	0.005
LDL-cholesterol, mg/dL	121 ± 36	123 ± 36	116 ± 34	0.020
FIB4 index	1.06 (0.77-1.44)	0.87 (0.67-1.07)	1.74 (1.46-2.20)	<0.001
Onset of DKD				
Decrease of eGFR <60, n (%)	182 (31.2)	106 (26.8)	76 (41.8)	0.001
Proteinuria (1+) or more, n (%)	178 (30.5)	112 (28.3)	66 (35.1)	0.090
DKD, n (%)	283 (48.5)	173 (43.7)	110 (58.5)	0.001
Observation period, years	6.0 (3.2-11.0)	6.0 (3.8-11.5)	5.0 (3.0-9.6)	0.013

Data is presented as mean ± SD, median (25-75th percentile), or percentages. DKD: diabetic kidney disease; DPP4: Dipeptidyl Peptidase-4, α GI: - α -Glucosidase Inhibitor; SGLT2: sodium glucose cotransporter 2. ACEi/ARB: Angiotensin-converting enzyme inhibitor and angiotensin II receptor blocker; HDL: high-density lipoprotein; LDL: low-density lipoprotein; UACR: urine albumin-to-creatinine ratio; eGFR: estimated glomerular filtration rate.

3.2. Univariate and multivariate analysis

The results of Kaplan-Meier survival analysis are shown in **Figure 2**. The median observation period was 6.0 (3.8 – 11.0) years. A total of 284 patients (incidence 48.6% [95%CI, 44.6%-52.7%], median 6.08 [3.88, 11.2] years) developed DKD, 182 (31.2% [27.8-35.3%], 8.72 [0.43, 12,6] years) patients developed DKD with eGFR <60 mL/min/1.73 m^2 and 178 (30.6% [95%CI, 27.0-34.4%], 8.12 [5.07, 13.2] years) with proteinuria. The risk of developing DKD was higher in FIB4 index >1.3 patients than in FIB4 index ≤1.3 patients (**Figure 2A**). Development of eGFR <60 mL/min/1.73 m^2 (**Figure 2B**) and proteinuria (**Figure 2C**) were also higher in FIB4 index >1.3 patients. As shown in **Figure 3**, FIB4 index >1.3 was a significant variable for the onset of DKD (hazard ratio [HR]: 1.68, 95% CI: 1.32-2.14, *P* < 0.001) (**Figure 3A**). Among other variables, baseline eGFR, baseline HbA1c and use of ACEi/ARB were significant risk variables for the onset of DKD. In the Cox proportional hazards model, adjusted for age, sex, BMI, baseline HbA1c, baseline eGFR, smoking and drinking status, comorbidities (hypertension, dyslipidemia), and anti-diabetic medications, FIB4 index >1.3 was a significant variable for the onset of DKD (adjusted HR: 1.54, 95% CI: 1.15-2.08, *P* =0.004). Because we could obtain HbA1c during follow-up only from one center (Fukushima Medical University), we calculated multivariate-adjusted analysis including median HbA1c during followup in a part of total participants (n=307, **Figure 4**): FIB4 index >1.3 and baseline eGFR were only a risk variable for the onset of DKD.

Development of proteinuria (**Figure 3C**) was also higher in the FIB4 index >1.3 patients (adjusted HR: 1.55, 95% CI: 1.08-2.23, *P* =0.020). Past smoker was another independent variable for proteinuria. Meanwhile, FIB4 index >1.3 was not a significant variable in the development of eGFR <60 mL/min/1.73 m^2 (**Figure 3B**). Instead, age, baseline eGFR, baseline HbA1c and use of sulfonylurea were risk variables for the development of eGFR <60.

We evaluated the impact of ultrasonography-determined NAFLD for the DKD hazard ratio in type 2 diabetic patients in whom abdominal ultrasonography could be performed (n=96). Multivariate analysis showed that the presence of NAFLD was not a significant predictor for onset of DKD (odds ratio 0.71; 95% CI, 0.37-1.36, P = 0.300).

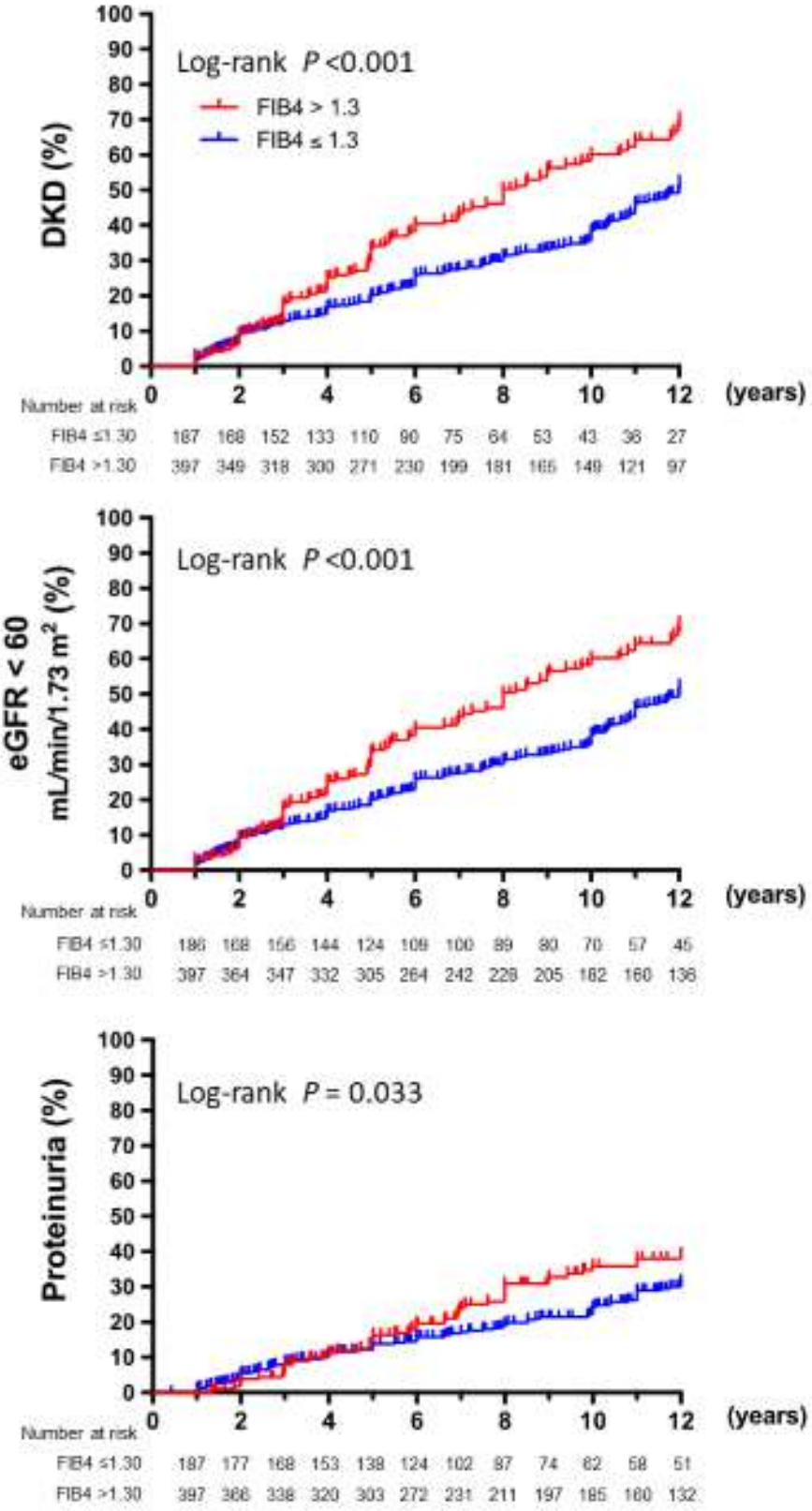
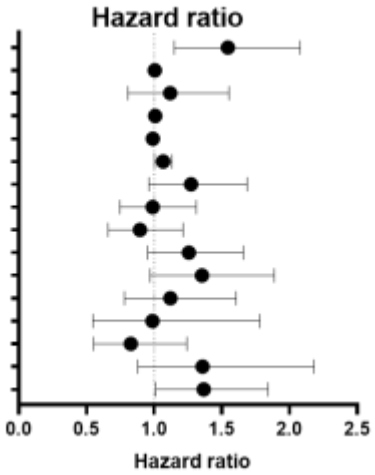


Figure 2. Kaplan Meier curves for the development of (A) diabetic kidney disease (DKD: eGFR < 60 mL/min/1.73 m^2 or proteinuria), (B) eGFR < 60 mL/min/1.73 m^2, and (C) proteinuria in type 2 diabetic patients with FIB4 index > 1.3 (red lines) or ≤ 1.3 (blue lines).

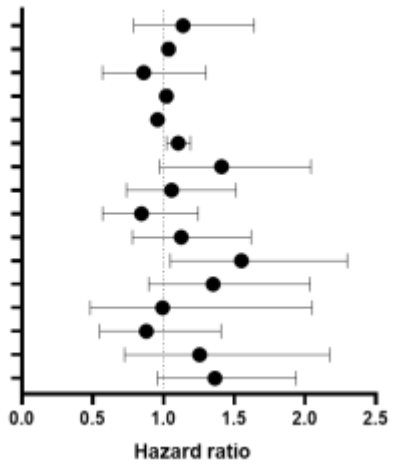
A. DKD

	Univariate	P value	Multivariate	P value
FIB4 > 1.3 (yes vs. no)	1.68 (1.32-2.14)	<0.001	1.54 (1.15-2.08)	0.004
Age (per year)	1.02 (1.01-1.03)	0.001	1.00 (0.99-1.02)	0.558
Sex (male vs. female)	1.19 (0.93-1.51)	0.147	1.12 (0.81-1.55)	0.485
BMI (kg/m^2)	1.00 (0.98-1.02)	0.897	1.01 (0.99-1.03)	0.480
eGFR (mL/min/1.73 m^2)	0.99 (0.98-0.99)	0.008	0.99 (0.98-0.99)	0.015
HbA1c (%)	1.05 (0.99-1.11)	0.062	1.07 (1.00-1.13)	0.041
Hypertension (yes vs. no)	1.49 (1.16-1.92)	0.002	1.27 (0.96-1.69)	0.087
Dyslipidemia (yes vs. no)	1.01 (0.77-1.32)	0.914	0.99 (0.75-1.31)	0.892
Past drinker (yes vs. no)	1.01 (0.80-1.27)	0.982	0.90 (0.66-1.21)	0.441
Past smoker (yes vs. no)	1.21 (0.96-1.53)	0.094	1.26 (0.95-1.66)	0.098
Sulfonylurea (yes vs. no)	1.52 (1.15-2.02)	0.003	1.35 (0.97-1.89)	0.069
Biguanide (yes vs. no)	1.19 (0.85-1.67)	0.318	1.12 (0.78-1.61)	0.549
Thiazolidine (yes vs. no)	1.11 (0.62-1.98)	0.725	0.99 (0.55-1.78)	0.953
αGI (yes vs. no)	1.09 (0.76-1.57)	0.633	0.83 (0.55-1.24)	0.382
Insulin (yes vs. no)	1.72 (1.13-2.62)	0.011	1.39 (0.88-2.18)	0.159
RAS inhibitor (yes vs. no)	1.62 (1.24-2.12)	<0.001	1.37 (1.01-1.84)	0.044



B. eGFR < 60

	Univariate	P value	Multivariate	P value
FIB4 > 1.3 (yes vs. no)	1.83 (1.37-2.47)	<0.001	1.14 (0.79-1.64)	0.437
Age (per year)	1.06 (1.04-1.07)	<0.001	1.04 (1.02-1.06)	<0.001
Sex (male vs. female)	0.80 (0.60-1.07)	0.154	0.86 (0.57-1.30)	0.409
BMI (kg/m^2)	1.00 (0.97-1.03)	0.923	1.02 (0.99-1.04)	0.119
eGFR (mL/min/1.73 m^2)	0.95 (0.94-0.97)	<0.001	0.96 (0.95-0.97)	<0.001
HbA1c (%)	1.06 (0.99-1.13)	0.081	1.10 (1.02-1.19)	0.008
Hypertension (yes vs. no)	1.90 (1.36-2.66)	<0.001	1.41 (0.97-2.04)	0.080
Dyslipidemia (yes vs. no)	1.09 (0.78-1.53)	0.589	1.06 (0.74-1.51)	0.643
Past drinker (yes vs. no)	0.70 (0.52-0.93)	0.013	0.84 (0.57-1.25)	0.477
Past smoker (yes vs. no)	0.84 (0.63-1.13)	0.278	1.13 (0.78-1.62)	0.585
Sulfonylurea (yes vs. no)	1.99 (1.43-2.77)	<0.001	1.55 (1.05-2.30)	0.037
Biguanide (yes vs. no)	1.64 (1.12-2.39)	0.010	1.35 (0.90-2.04)	0.133
Thiazolidine (yes vs. no)	1.20 (0.59-2.44)	0.620	0.99 (0.48-2.05)	0.965
αGI (yes vs. no)	1.31 (0.85-2.01)	0.224	0.88 (0.55-1.41)	0.486
Insulin (yes vs. no)	1.74 (1.05-2.86)	0.031	1.26 (0.73-2.17)	0.416
RAS inhibitor (yes vs. no)	1.94 (1.42-2.65)	<0.001	1.36 (0.96-1.94)	0.066



C. Proteinuria

	Univariate	P value	Multivariate	P value
FIB4 > 1.3 (yes vs. no)	1.39 (1.02-1.88)	0.035	1.55 (1.08-2.23)	0.020
Age (per year)	1.00 (0.98-1.01)	0.518	0.99 (0.97-1.01)	0.371
Sex (male vs. female)	1.64 (1.19-2.26)	0.002	1.30 (0.86-1.98)	0.196
BMI (kg/m^2)	1.01 (0.98-1.03)	0.561	1.01 (0.98-1.03)	0.565
eGFR (mL/min/1.73 m^2)	1.01 (1.00-1.01)	0.026	1.00 (0.99-1.01)	0.222
HbA1c (%)	1.09 (1.02-1.16)	0.013	1.07 (0.99-1.16)	0.087
Hypertension (yes vs. no)	1.40 (1.01-1.92)	0.042	1.18 (0.83-1.68)	0.326
Dyslipidemia (yes vs. no)	0.98 (0.71-1.37)	0.931	0.98 (0.69-1.39)	0.794
Past drinker (yes vs. no)	1.28 (0.95-1.73)	0.098	0.98 (0.68-1.41)	0.828
Past smoker (yes vs. no)	1.74 (1.29-2.34)	<0.001	1.47 (1.03-2.09)	0.027
Sulfonylurea (yes vs. no)	1.56 (1.11-2.20)	0.011	1.35 (0.90-2.02)	0.123
Biguanide (yes vs. no)	1.17 (0.77-1.77)	0.464	1.14 (0.73-1.77)	0.609
Thiazolidine (yes vs. no)	1.75 (0.95-3.23)	0.073	1.78 (0.95-3.33)	0.078
αGI (yes vs. no)	1.09 (0.70-1.71)	0.709	0.81 (0.49-1.34)	0.483
Insulin (yes vs. no)	1.51 (0.89-2.57)	0.125	1.24 (0.70-2.19)	0.463
RAS inhibitor (yes vs. no)	1.58 (1.13-2.20)	0.008	1.39 (0.95-2.03)	0.102

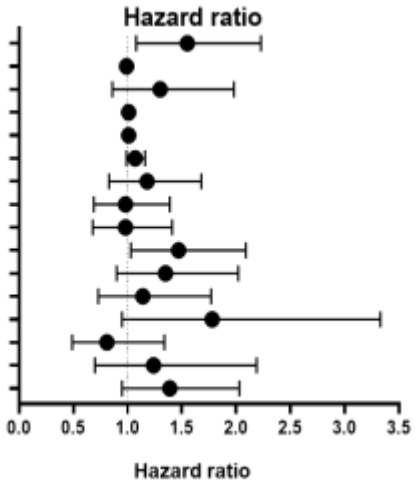


Figure 3. Univariate and Cox proportional hazard ratios of FIB4 index > 1.3 for the development of (A) diabetic kidney disease (DKD: eGFR < 60 mL/min/1.73 m^2 or proteinuria), (B) eGFR < 60 mL/min/1.73 m^2, and (C) proteinuria in type 2 diabetic patients. Cox proportional hazard models were adjusted for age, sex, BMI, baseline HbA1c, baseline eGFR, smoking and drinking status (current or past), comorbidities (hypertension, dyslipidemia) and anti-diabetic and anti-hypertensive medications. 95% CI, 95% confidence interval.

	Univariate	P value	Multivariate	P value
FIB4 > 1.3 (yes vs. no)	2.00 (1.45-2.76)	<0.001	1.72 (1.16-2.56)	0.007
Age (per year)	1.02 (1.01-1.04)	0.003	1.01 (0.99-1.03)	0.529
Sex (male vs. female)	1.13 (0.84-1.52)	0.418	1.06 (0.71-1.59)	0.740
BMI (kg/m^2)	0.99 (0.97-1.02)	0.567	1.00 (0.98-1.03)	0.791
eGFR (mL/min/1.73 m^2)	0.98 (0.97-0.99)	<0.01	0.99 (0.98-0.99)	0.014
Median HbA1c (%)	0.97 (0.81-1.17)	0.777	1.06 (0.85-1.32)	0.618
Hypertension (yes vs. no)	1.28 (0.91-1.80)	0.161	1.03 (0.69-1.55)	0.891
Dyslipidemia (yes vs. no)	0.96 (0.69-1.34)	0.808	0.93 (0.66-1.32)	0.684
Past drinker (yes vs. no)	1.00 (0.74-1.34)	0.977	0.90 (0.62-1.31)	0.561
Past smoker (yes vs. no)	1.06 (0.79-1.42)	0.716	1.07 (0.74-1.54)	0.731
Sulfonylurea (yes vs. no)	1.22 (0.88-1.69)	0.232	1.08 (0.73-1.60)	0.710
Biguanide (yes vs. no)	1.17 (0.78-1.75)	0.445	1.13 (0.73-1.74)	0.581
Thiazolidine (yes vs. no)	1.33 (0.70-2.52)	0.385	1.36 (0.70-2.64)	0.365
αGI (yes vs. no)	0.99 (0.68-1.44)	0.946	0.83 (0.54-1.25)	0.368
Insulin (yes vs. no)	1.68 (1.10-2.58)	0.016	1.49 (0.94-2.36)	0.090
RAS inhibitor (yes vs. no)	1.53 (1.12-2.08)	0.007	1.35 (0.95-1.91)	0.098

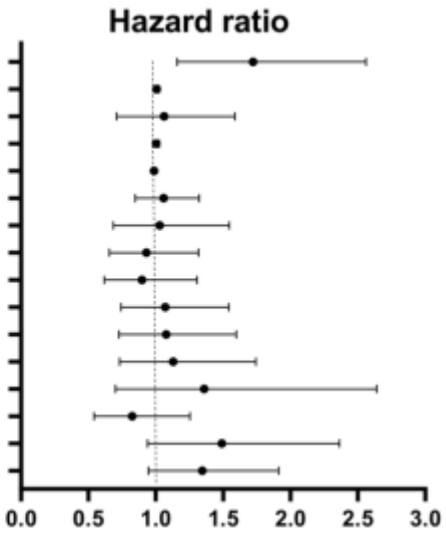


Figure 4. Hazard ratios for the development of diabetic kidney disease (DKD: eGFR < 60mL/min/1.73 m2 or proteinuria in type 2 diabetic patients (Cox proportional hazards model). 95% CI, 95%confidence interval. In this model, median HbA1c (%) was used inplace of baseline HbA1c in **Figure 3A**.

3.3. Sensitivity analysis

For sensitivity analyses: univariate and Cox proportional hazards models were repeated: 1) by using the FIB4 index as continuous (**Figure 5**) or quartile variables (**Figure 6**); 2) by HbA1c as a time dependent covariate plus possible emerging biomarker for DKD (white blood cell count) (**Figure 7**); 3) by a new data-set with multiple imputation method for missing data analysis (**Figure 8 and 9**): 4) time dependent ROC of FIB4 index for development of DKD, eGFR and proteinuria (**Figure 10**).

In univariate and Cox proportional hazards models, (**Figure 5**) or quartile variables (**Figure 6**) the FIB4 index was a significant variable for the onset of DKD and proteinuria. When HbA1c during follow-up added as a time dependent covariate, FIB4 index >1.3 was still a significant variable for the onset of DKD and proteinuria. Missing pattern of variables was shown in **Figure 8**. Duration of diabetes was frequent and missed mostly in one center (Tomishiro Central Hospital). The other variables were considered to be Missing Completely at Random (MCAR)(Little's test for MCAR, p=1.000). We therefore used only Fukushima Medical University database in the model including diabetes duration (**Figure 7**). On a new data-set with multiple imputation method for missing data analysis, FIB4 index was also a significant variable for the onset of DKD and proteinuria. (**Figure 9**). Finally, we performed time dependent receiver operating characteristic curve (ROC) analysis of FIB4 index for development of DKD, eGFR and proteinuria (**Figure 10**). The addition of FIB4 index in the classical risk model for the development of DKD and proteinuria significantly improved at 10 years, but not at 5 and 7 years. Annual changes in FIB4 index during the observational periods were shown in **Figure 11.** The values of FIB4 index gradually increased in the ≤1.3 and >1.3 groups during over 10 years, but the value range of 25% to 75% ranges did not cross between groups (**Figure 11**). E-values, relative risk +√(relative risk (relative risk-1), for DKD and proteinuria were 1.89 and 1.83, respectively, indicating unmeasured confounding variables with hazard ratios over these values may affect the impact of FIB4 index.

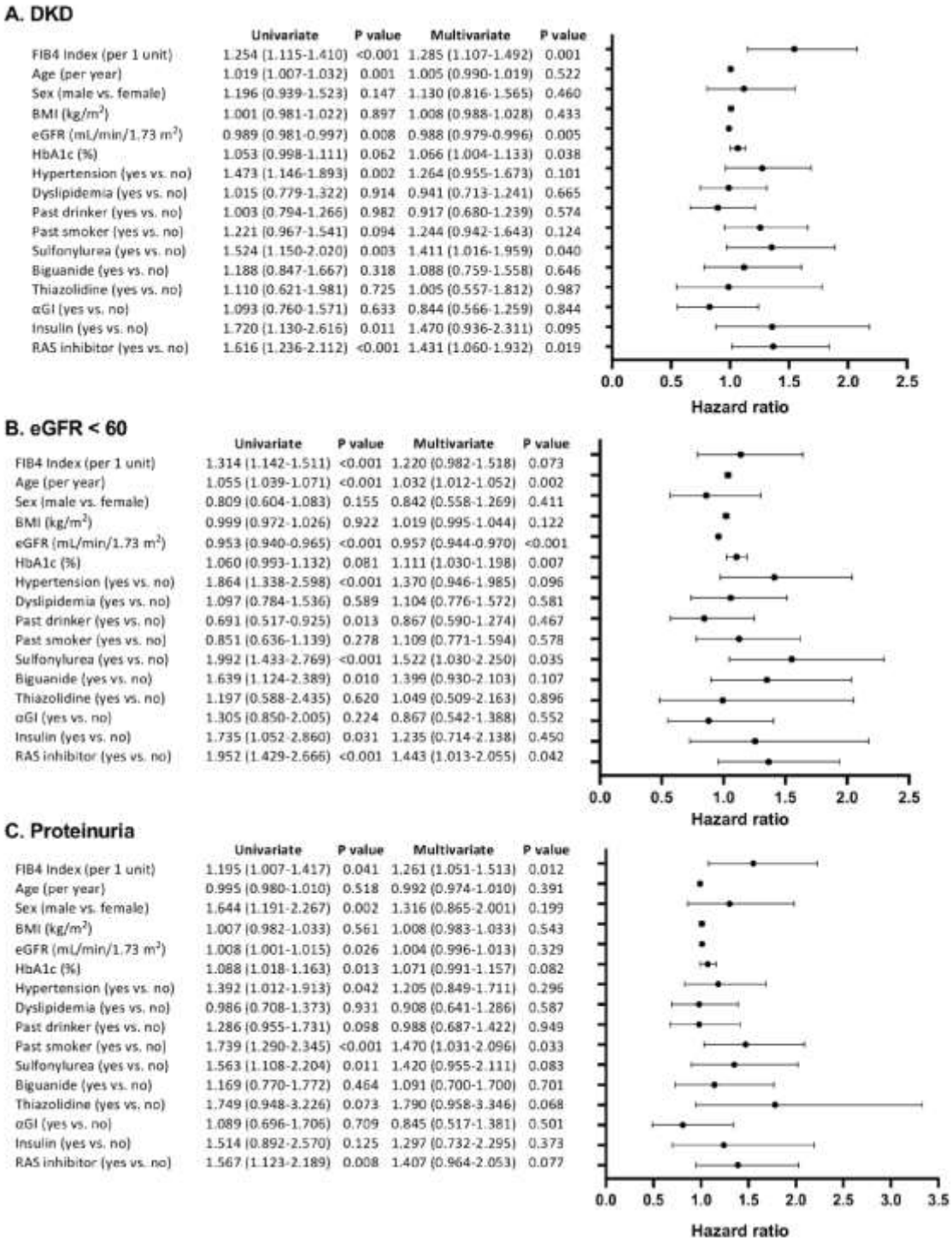
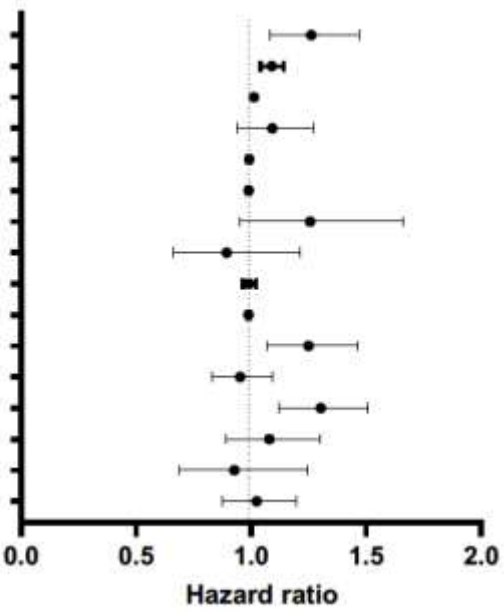


Figure 5. Univariate and Cox proportional hazard ratios of FIB4 index (per 1 unit) for the development of (A) diabetic kidney disease (DKD: eGFR < 60 mL/min/1.73 m2 or proteinuria), (B) eGFR < 60 mL/min/1.73 m2, and (C) proteinuria in type 2 diabetic patients. Cox proportional hazard models were adjusted for age, sex, BMI, baseline HbA1c, baseline eGFR, smoking and drinking status (current or past), comorbidities (hypertension, dyslipidemia) and anti-diabetic and anti-hypertensive medications. 95% CI, 95% confidence interval.

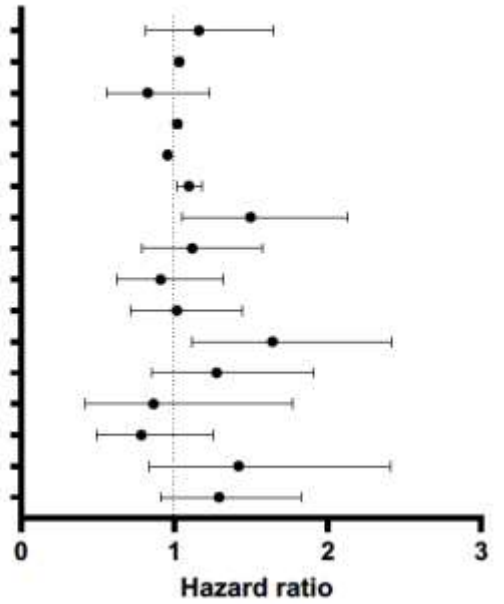
A. DKD

	Multivariate	P value
FIB4 Quartile	1.202(1.051-1.374)	0.007
Age (per year)	1.003(0.988-1.018)	0.707
Sex (male vs. female)	1.114(0.805-1.542)	0.514
BMI (kg/m2)	1.008(0.989-1.028)	0.407
eGFR (mL/min/1.73 m2)	0.990(0.981-0.998)	0.017
HbA1c (%)	1.066(1.003-1.132)	0.039
Hypertension (yes vs. no)	1.273(0.962-1.686)	0.092
Dyslipidemia (yes vs. no)	0.969(0.732-1.281)	0.824
Past drinker (yes vs. no)	0.905(0.67-1.224)	0.518
Past smoker (yes vs. no)	1.256(0.952-1.658)	0.107
Sulfonylurea (yes vs. no)	1.395(1.004-1.939)	0.047
Biganide (yes vs. no)	1.086(0.758-1.557)	0.653
Thiazolidine (yes vs. no)	0.978(0.543-1.762)	0.94
αGI (yes vs. no)	0.847(0.568-1.264)	0.416
Insulin (yes vs. no)	1.42(0.901-2.237)	0.13
RAS Inhibitor (yes vs. no)	1.40(1.039-1.887)	0.027



B. eGFR < 60

	Multivariate	P value
FIB4 Quartile	1.109(0.937-1.314)	0.230
Age (per year)	1.033(1.012-1.054)	0.002
Sex (male vs. female)	0.834(0.553-1.259)	0.388
BMI (kg/m2)	1.019(0.996-1.044)	0.112
eGFR (mL/min/1.73 m2)	0.958(0.945-0.971)	<0.001
HbA1c (%)	1.109(1.028-1.196)	0.007
Hypertension (yes vs. no)	1.399(0.966-2.025)	0.075
Dyslipidemia (yes vs. no)	1.092(0.767-1.555)	0.625
Past drinker (yes vs. no)	0.859(0.583-1.267)	0.444
Past smoker (yes vs. no)	1.117(0.776-1.609)	0.552
Sulfonylurea (yes vs. no)	1.522(1.028-2.252)	0.036
Biganide (yes vs. no)	1.390(0.922-2.094)	0.116
Thiazolidine (yes vs. no)	1.013(0.492-2.086)	0.971
αGI (yes vs. no)	0.845(0.528-1.352)	0.482
Insulin (yes vs. no)	1.233(0.71-2.142)	0.457
RAS Inhibitor (yes vs. no)	1.402(0.987-1.991)	0.059



C. Proteinuria

	Multivariate	P value
FIB4 Quartile	1.246(1.058-1.469)	0.009
Age (per year)	0.988(0.969-1.007)	0.207
Sex (male vs. female)	1.299(0.857-1.969)	0.218
BMI (kg/m2)	1.008(0.983-1.034)	0.518
eGFR (mL/min/1.73 m2)	1.005(0.997-1.014)	0.201
HbA1c (%)	1.071(0.992-1.156)	0.081
Hypertension (yes vs. no)	1.176(0.827-1.672)	0.366
Dyslipidemia (yes vs. no)	0.958(0.675-1.36)	0.81
Past drinker (yes vs. no)	0.971(0.674-1.397)	0.873
Past smoker (yes vs. no)	1.493(1.047-2.127)	0.027
Sulfonylurea (yes vs. no)	1.400(0.943-2.08)	0.095
Biganide (yes vs. no)	1.112(0.713-1.736)	0.639
Thiazolidine (yes vs. no)	1.786(0.957-3.333)	0.069
αGI (yes vs. no)	0.868(0.531-1.42)	0.573
Insulin (yes vs. no)	1.251(0.706-2.219)	0.443
RAS Inhibitor (yes vs. no)	1.433(0.98-2.095)	0.063

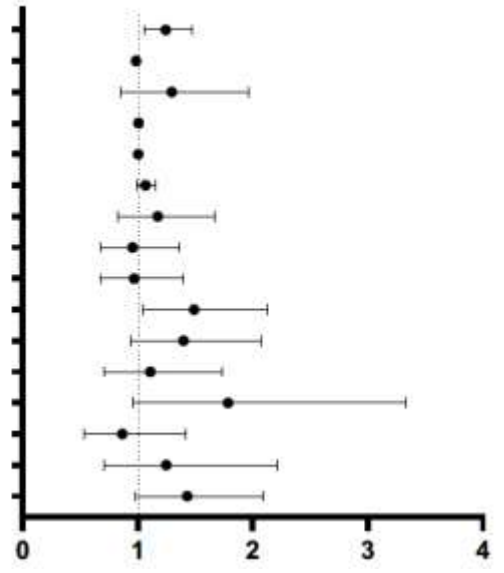
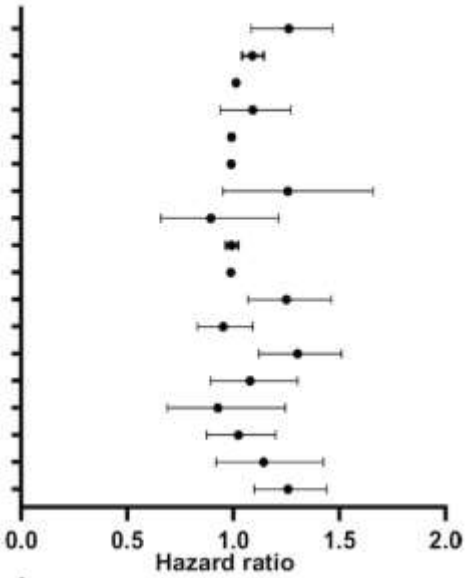


Figure 6. Cox proportional hazard ratios of FIB4 quartile for the development of (A) diabetic kidney disease (DKD: eGFR < 60 mL/min/1.73 m2 or proteinuria), (B) eGFR < 60 mL/min/1.73 m2, and (C) proteinuria in type 2 diabetic patients. Cox proportional hazard models were adjusted for age, sex, BMI, baseline HbA1c, baseline eGFR, smoking and drinking status (current or past), comorbidities (hypertension, dyslipidemia) and anti-diabetic and anti-hypertensive medications. 95% CI, 95% confidence interval.

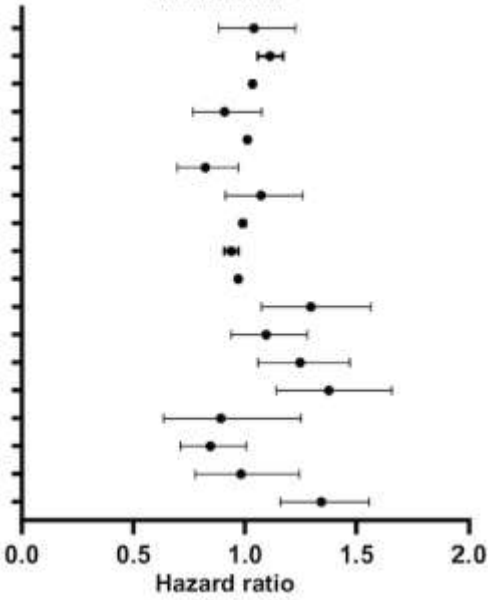
A. DKD

	Multivariate	P value
FIB4 > 1.3 (yes vs. no)	1.262 (1.085-1.468)	0.0025
HbA1c time dependent covariate (%)	1.091 (1.042-1.143)	<0.001
Age (per year)	1.013 (1.006-1.021)	<0.001
Sex (male vs. female)	1.093 (0.939-1.271)	0.252
BMI (kg/m^2)	0.992 (0.980-1.003)	0.140
Past drinker (yes vs. no)	0.827 (0.714-0.959)	0.012
Past smoker (yes vs. no)	0.990 (0.858-1.141)	0.886
Duration of diabetes (years)	0.981 (0.968-0.993)	0.0027
White blood cell ($10^3/\mu L$)	0.993 (0.965-1.022)	0.625
eGFR (mL/min/1.73 m^2)	0.989 (0.985-0.993)	<0.001
Hypertension (yes vs. no)	1.251 (1.070-1.463)	0.0051
Dyslipidemia (yes vs. no)	0.953 (0.831-1.093)	0.492
Sulfonylurea (yes vs. no)	1.303 (1.123-1.511)	<0.001
Biganide (yes vs. no)	1.080 (0.894-1.304)	0.427
Thiazolidine (yes vs. no)	0.928 (0.690-1.246)	0.617
αGI (yes vs. no)	1.025 (0.875-1.200)	0.759
Insulin (yes vs. no)	1.144 (0.919-1.424)	0.228
RAS Inhibitor (yes vs. no)	1.259 (1.098-1.442)	<0.001



B. eGFR < 60

	Multivariate	P value
FIB4 > 1.3 (yes vs. no)	1.041 (0.883-1.227)	0.635
HbA1c time dependent covariate (%)	1.113 (1.058-1.172)	<0.001
Age (per year)	1.035 (1.026-1.044)	<0.001
Sex (male vs. female)	0.909 (0.767-1.077)	0.269
BMI (kg/m^2)	1.012 (1.003-1.022)	0.01
Past drinker (yes vs. no)	0.823 (0.696-0.972)	0.022
Past smoker (yes vs. no)	1.073 (0.913-1.260)	0.394
Duration of diabetes (years)	0.991 (0.978-1.004)	0.182
White blood cell ($10^3/\mu L$)	0.939 (0.908-0.972)	<0.001
eGFR (mL/min/1.73 m^2)	0.971 (0.966-0.976)	<0.001
Hypertension (yes vs. no)	1.297 (1.076-1.565)	0.0065
Dyslipidemia (yes vs. no)	1.096 (0.938-1.280)	0.249
Sulfonylurea (yes vs. no)	1.248 (1.059-1.471)	0.0084
Biganide (yes vs. no)	1.377 (1.142-1.659)	<0.001
Thiazolidine (yes vs. no)	0.893 (0.636-1.253)	0.513
αGI (yes vs. no)	0.846 (0.711-1.008)	0.061
Insulin (yes vs. no)	0.983 (0.777-1.245)	0.89
RAS Inhibitor (yes vs. no)	1.344 (1.160-1.558)	<0.001



C. Proteinuria

	Multivariate	P value
FIB4 > 1.3 (yes vs. no)	1.252 (1.027-1.526)	0.026
HbA1c time dependent covariate (%)	1.055 (0.997-1.117)	0.062
Age (per year)	1.003 (0.994-1.013)	0.503
Sex (male vs. female)	1.043 (0.850-1.280)	0.685
BMI (kg/m^2)	0.978 (0.962-0.995)	0.013
Past drinker (yes vs. no)	0.834 (0.689-1.009)	0.061
Past smoker (yes vs. no)	1.120 (0.924-1.357)	0.247
Duration of diabetes (years)	0.984 (0.968-1.000)	0.053
White blood cell ($10^3/\mu L$)	1.080 (1.046-1.117)	<0.001
eGFR (mL/min/1.73 m^2)	1.001 (0.996-1.005)	0.837
Hypertension (yes vs. no)	1.274 (1.030-1.575)	0.026
Dyslipidemia (yes vs. no)	0.731 (0.611-0.875)	<0.001
Sulfonylurea (yes vs. no)	1.882 (1.554-2.279)	<0.001
Biganide (yes vs. no)	0.895 (0.694-1.154)	0.39
Thiazolidine (yes vs. no)	1.699 (1.256-2.297)	<0.001
αGI (yes vs. no)	1.196 (0.978-1.462)	0.081
Insulin (yes vs. no)	1.134 (0.870-1.479)	0.352
RAS Inhibitor (yes vs. no)	1.564 (1.307-1.872)	<0.001

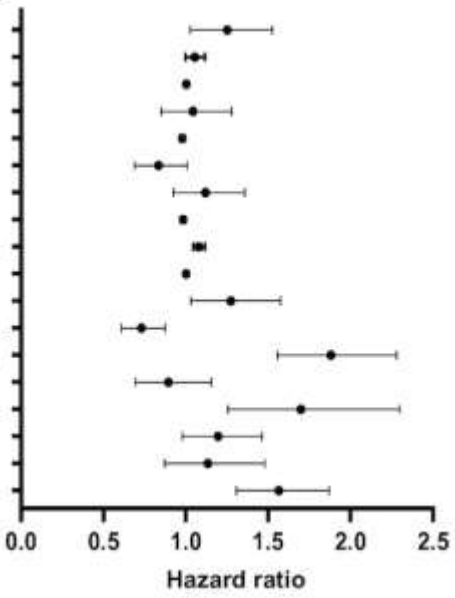


Figure 7. Cox proportional hazard ratios of FIB4 index > 1.3 for the development of (A) diabetic kidney disease (DKD: eGFR < 60 mL/min/1.73 m^2 or proteinuria), (B) eGFR < 60 mL/min/1.73 m^2, and (C) proteinuria in type 2 diabetic patients. Cox proportional hazard models were adjusted for age, sex, BMI, a HbA1c time dependent covariate, baseline eGFR, smoking and drinking status (current or past), comorbidities (hypertension, dyslipidemia) and anti-diabetic and anti-hypertensive medications. 95% CI, 95% confidence interval. N=318

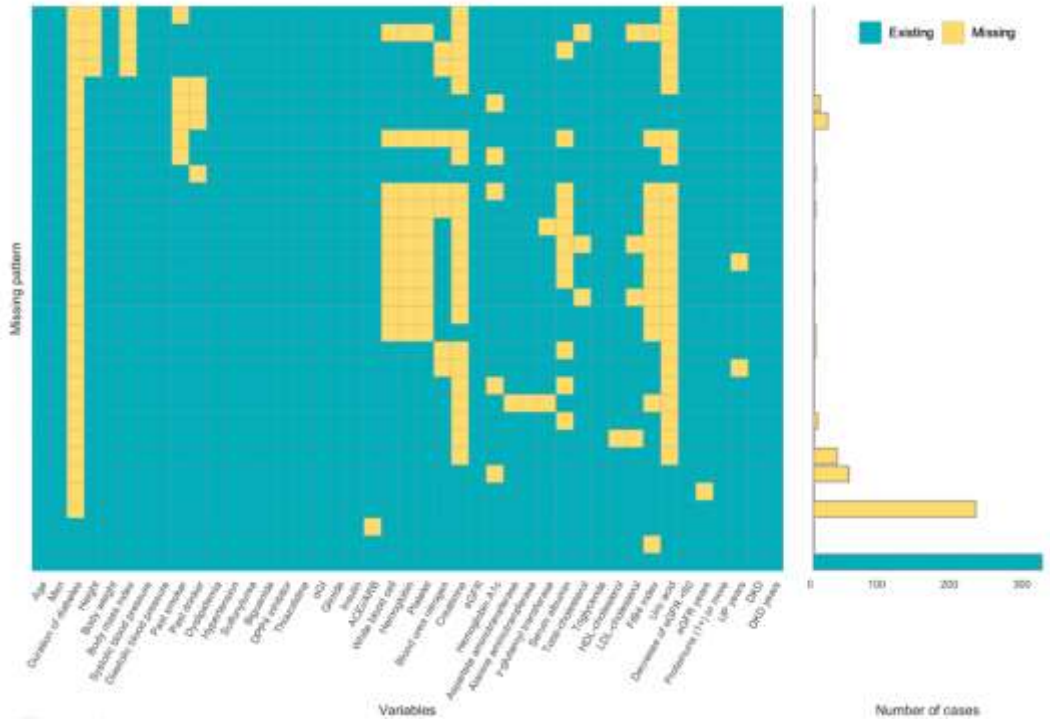
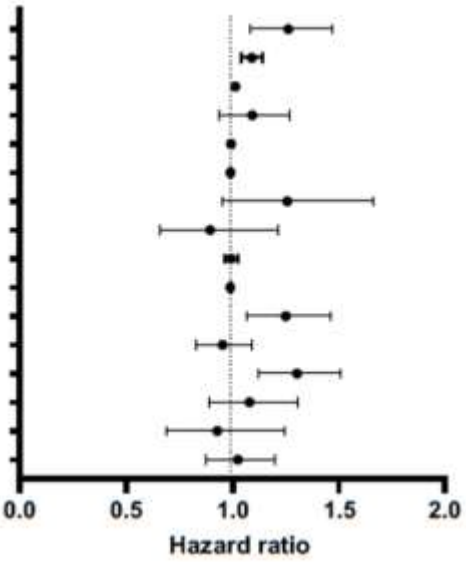


Figure 8. Pattern of missing data. In the left panel, green and yellow represents missing and existing values in the dataset. Each row represents the missing pattern. In the right panel, the horizontal bars display the frequencies of the corresponding combinations of existing and missing variables. VIM package 5.1.1 and ggplot 2.3.3 running on R 3.6.3 were used for visualization of the missing pattern.

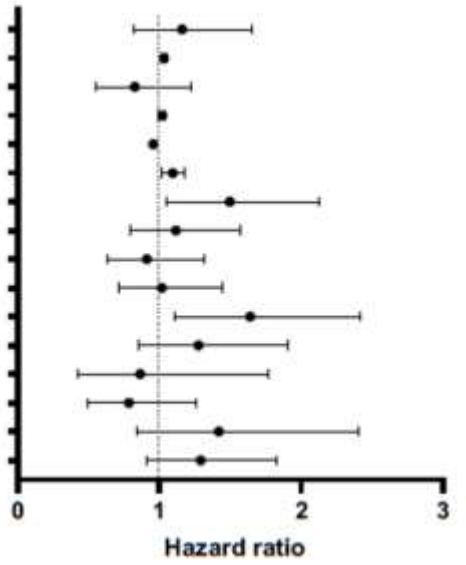
A. DKD

	Multivariate	P value
FIB4 Index > 1.3 (yes vs. no)	1.513(1.139-2.011)	0.006
Age (per year)	1.003(0.989-1.018)	0.668
Sex (male vs. female)	1.132(0.825-1.552)	0.451
BMI (kg/m2)	1.009(0.989-1.029)	0.369
eGFR (mL/min/1.73 m2)	0.991(0.983-0.999)	0.036
HbA1c (%)	1.045(0.986-1.107)	0.146
Hypertension (yes vs. no)	1.323(1.014-1.726)	0.039
Dyslipidemia (yes vs. no)	1.009(0.773-1.319)	0.946
Past drinker (yes vs. no)	0.91(0.681-1.216)	0.537
Past smoker (yes vs. no)	1.159(0.888-1.51)	0.306
Sulfonylurea (yes vs. no)	1.437(1.037-1.991)	0.030
Biganide (yes vs. no)	1.027(0.72-1.464)	0.886
Thiazolidine (yes vs. no)	0.805(0.459-1.412)	0.449
αGI (yes vs. no)	0.78(0.524-1.163)	0.223
Insulin (yes vs. no)	1.559(1.002-2.425)	0.050
RAS Inhibitor (yes vs. no)	1.267(0.945-1.698)	0.115



B. eGFR < 60

	Multivariate	P value
FIB4 Index > 1.3 (yes vs. no)	1.16(0.815-1.652)	0.424
Age (per year)	1.033(1.014-1.053)	0.001
Sex (male vs. female)	0.827(0.557-1.228)	0.353
BMI (kg/m2)	1.02(0.996-1.045)	0.110
eGFR (mL/min/1.73 m2)	0.956(0.943-0.969)	0.000
HbA1c (%)	1.094(1.017-1.177)	0.016
Hypertension (yes vs. no)	1.498(1.051-2.134)	0.025
Dyslipidemia (yes vs. no)	1.118(0.793-1.575)	0.525
Past drinker (yes vs. no)	0.912(0.629-1.322)	0.630
Past smoker (yes vs. no)	1.017(0.716-1.445)	0.826
Sulfonylurea (yes vs. no)	1.641(1.114-2.42)	0.012
Biganide (yes vs. no)	1.276(0.852-1.91)	0.238
Thiazolidine (yes vs. no)	0.865(0.423-1.772)	0.692
αGI (yes vs. no)	0.785(0.491-1.253)	0.310
Insulin (yes vs. no)	1.42(0.837-2.409)	0.195
RAS Inhibitor (yes vs. no)	1.291(0.913-1.826)	0.149



C. Proteinuria

	Multivariate	P value
FIB4 Index > 1.3 (yes vs. no)	1.489(1.049-2.112)	0.031
Age (per year)	0.992(0.974-1.01)	0.386
Sex (male vs. female)	1.279(0.85-1.924)	0.238
BMI (kg/m2)	1.011(0.986-1.036)	0.401
eGFR (mL/min/1.73 m2)	1.008(1.000-1.016)	0.050
HbA1c (%)	1.049(0.974-1.13)	0.218
Hypertension (yes vs. no)	1.213(0.868-1.697)	0.260
Dyslipidemia (yes vs. no)	0.993(0.709-1.391)	0.969
Past drinker (yes vs. no)	1.002(0.703-1.429)	0.859
Past smoker (yes vs. no)	1.406(1.000-1.977)	0.053
Sulfonylurea (yes vs. no)	1.401(0.94-2.087)	0.098
Biganide (yes vs. no)	1.05(0.674-1.636)	0.831
Thiazolidine (yes vs. no)	1.496(0.825-2.71)	0.185
αGI (yes vs. no)	0.844(0.517-1.377)	0.497
Insulin (yes vs. no)	1.34(0.763-2.353)	0.310
RAS Inhibitor (yes vs. no)	1.313(0.906-1.902)	0.152

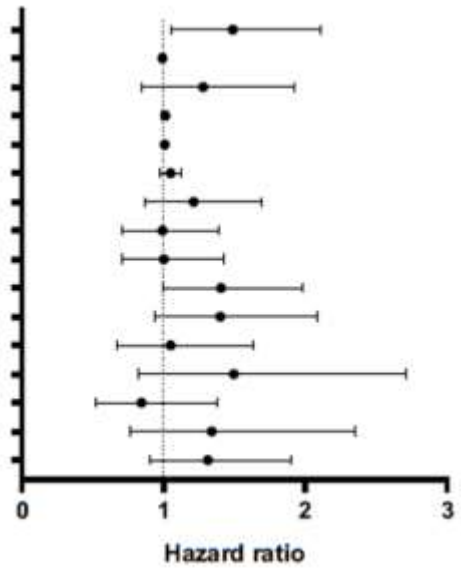


Figure 9. Cox proportional hazard ratios of FIB4 index >1.30 for the development of (A) diabetic kidney disease (DKD: eGFR < 60 mL/min/1.73 m2 or proteinuria), (B) eGFR < 60 mL/min/1.73 m2, and (C) proteinuria in type 2 diabetic patients of a new virtual database by multiple imputation method for missing data. Cox proportional hazard models were adjusted for age, sex, BMI, baseline HbA1c, baseline eGFR, smoking and drinking status (current or past), comorbidities (hypertension, dyslipidemia) and anti-diabetic and anti-hypertensive medications. 95% CI, 95% confidence interval. n=691

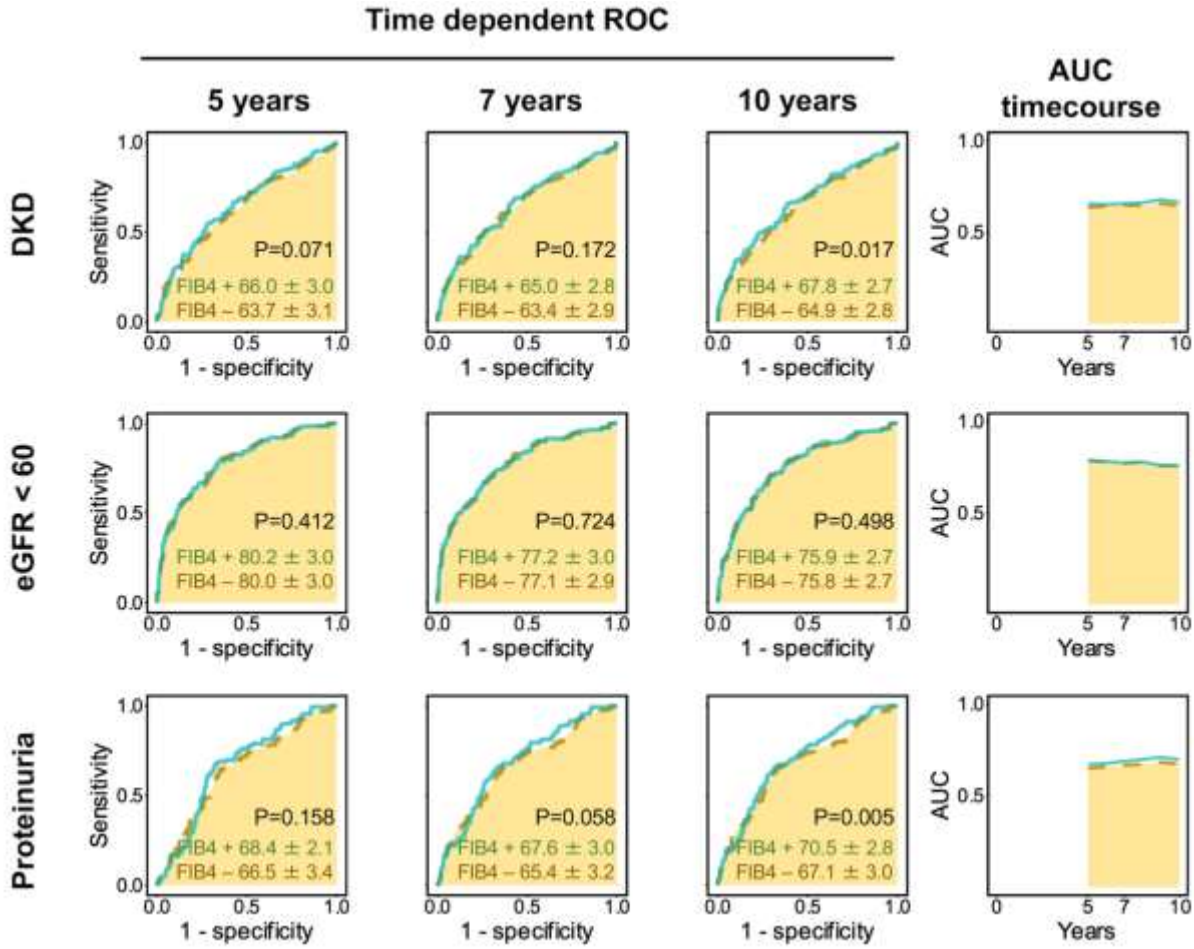
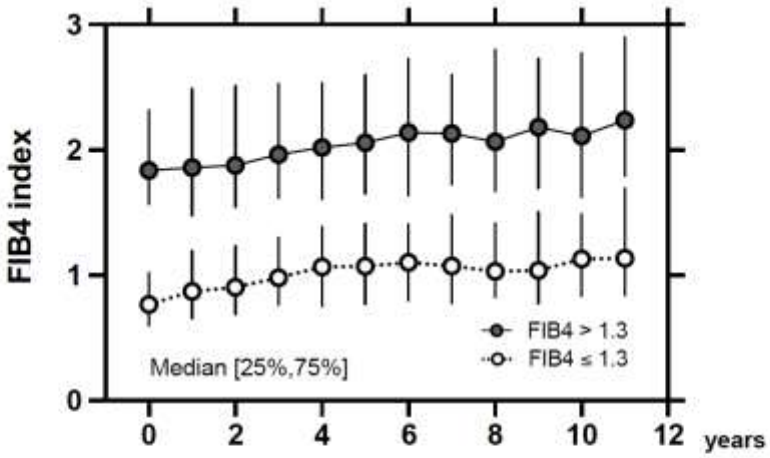


Figure 10. Time dependent receiver operating characteristic curve (ROC) analysis of FIB4 index for development of DKD, eGFR and proteinuria. Models included with or without FIB4 index were analyzed by area under the curve (AUC) of ROC analysis. Model covariants except FIB4 index included age, sex, BMI, baseline HbA1c, baseline eGFR, smoking and drinking status (current or past), comorbidities (hypertension, dyslipidemia) and anti-diabetic and anti-hypertensive medications. P values were for comparisons between model with or without FIB4 index.



		Baseline	1	2	3	4	5	6	7	8	9	10	11
FIB4 >1.30	Median	1.84	1.86	1.88	1.96	2.02	2.06	2.14	2.13	2.06	2.18	2.11	2.24
	25%	1.57	1.48	1.55	1.62	1.61	1.65	1.64	1.72	1.67	1.69	1.62	1.79
	75%	2.32	2.49	2.51	2.53	2.53	2.60	2.73	2.60	2.80	2.73	2.77	2.90
FIB4 ≤1.30	Median	0.77	0.87	0.90	0.98	1.07	1.07	1.10	1.07	1.03	1.04	1.13	1.13
	25%	0.60	0.65	0.69	0.76	0.75	0.77	0.80	0.78	0.82	0.78	0.83	0.84
	75%	1.02	1.20	1.23	1.30	1.38	1.41	1.41	1.48	1.41	1.50	1.48	1.69

Figure 11. Annual changes in FIB4 index during the observational periods in FIB4 index >1.3 or ≤1.3. Values are median [25%, 75%].

4. Discussion

In this study, we investigated the impact of the FIB4 index >1.3 on the development of DKD in Japanese type 2 diabetic patients and obtained two major findings. First, the group with the FIB4 index >1.3 showed an increased DKD by Cox proportional HR and in the Kaplan-Meier curve. Second, the FIB4 index >1.3 was associated with the development of proteinuria, but not with eGFR <60. For the first time, this study demonstrated that the FIB4 index >1.3, an index of liver fibrosis, has a prognostic impact on development of CKD, particularly on that of proteinuria, in Japanese type 2 diabetic patients.

Previous reports showed that the FIB4 index predicts onset of CKD in non-diabetic patients [22-25]. However, the prognostic impact of the FIB4 index in DKD remained unclarified. For the first time, this study exhibited that an FIB4 index >1.3 was associated with onset of DKD in type 2 diabetic patients. Based on a study evaluating the utility of the FIB4 index as a marker of advanced fibrosis (bridging fibrosis or cirrhosis) in NAFLD, an FIB4 index ≥2.67 had an 80% positive predictive value and an FIB4 index ≤1.30 had a 90% negative predictive value [16]. Therefore, two groups, ≤1.3 and >1.3 of FIB4 index, can be estimated as a group either excluded or not excluded for advanced liver fibrosis in type 2 diabetic patients. To elucidate which of liver fibrosis or fatty liver is crucial for DKD development, we evaluated the impact of ultrasonography-determined NAFLD for the DKD hazard ratio in type 2 diabetic patients in whom abdominal ultrasonography could be performed (n=96). Multivariate analysis showed that the presence of NAFLD was not a significant predictor for onset of DKD. Onnerhag et al. reported that the stage of fibrosis, diagnosed by liver biopsy, was strongly correlated with the FIB4 index, and that the FIB4 index is a better predictor for metabolic complications, including CKD [18]. Collectively, the presence of liver fibrosis, but not the presence or absence of NAFLD, can be well correlated with the development of DKD. We calculated the optimal cutoff point of FIB4 index by the highest Youden index for developing DKD, eGFR <60 mL/min/1.73 m^2 and proteinuria (EZR 1.40)[26](**Table 2**). Interestingly, the optimal cutoff points of FIB4 index for predicting development of DKD, eGFR <60 and proteinuria were 1.296, 1.095 and 1.197, respectively, close to the cutoff (≤1.3) in the current study. This may support the notion that the exclusion of liver fibrosis is useful to predict delaying the onset of DKD.

Table 2. The optimal cutoff point of FIB4 index by the highest Youden index for diabetic kideny disease (DKD)

	FIB4 index cutoff	Sensitivity	Specificity	AUC
DKD	1.296	0.398	0.740	0.566 (0.520-0.613)
eGFR < 60 mL/min/1.73 m^2	1.095	0.595	0.586	0.603 (0.554-0.651)
Proteinuria +	1.197	0.461	0.638	0.528 (0.475-0.581)

The optimal cutoff point of FIB4 index by the highest Youden index (EZR 1.40, Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. Bone Marrow Transplant 2013; 48: 452-458, doi:10.1038/bmt.2012.244). Interestingly, the optimal cutoff points of FIB4 index especially for DKD were close to our cut off point of FIB4 index <1.3. We therefore determined to use this value and discussed as follows.

In this study, an FIB4 index of >1.3 was also correlated with the development of proteinuria, but not an eGFR of <60. Yilmaz et al. reported that patients with microalbuminuria had a higher rate of fibrosis among non-diabetic patients with NAFLD [27]. Yeung et al. reported that liver

fibrosis, but not liver steatosis, is associated with albuminuria in Chinese patients with type 2 diabetes [28]. These results agree with ours. On the other hand, our study showed no significant correlation between the FIB4 index and an eGFR <60. In contrast, Onnerhag et al. reported a significant correlation between a high FIB4 index and the onset of eGFR <60 in 144 Swedish NAFLD patients (included 22% diabetes mellitus) [18]. Although we cannot explain the discrepancy between our study and the one completed by Onnerhag et al., there might be different pathophysiological background between liver fibrosis and CKD with or without diabetes, as discussed below.

Two potential underlying mechanisms regarding why high values of the FIB4 index were linked to the development of DKD are discussed.

First, liver fibrosis, estimated by FIB4 index, might not be causal and could be a simple correlation to onset of CKD. Based on formula [age (year) × AST (IU/L) / (√ALT (IU/L) × platelet count (10^9/L))] [29], the FIB4 index can increase either by aging and an increase in AST to ALT ratio or by a decrease in platelet count. In patients with liver fibrosis, inhibition of thrombopoietin synthesis [30], enhancement of platelets uptake by the liver with or without attendant splenomegaly can cause thrombocytopenia [31]. Currently, evidence lacks that thrombocytopenia is causally linked to the onset of CKD, at least an earlier stage of CKD [32]. In contrast, an increase in AST to ALT ratio could be elicited by metabolic derangements often observed in aging, diabetes mellitus/glucose intolerance/insulin resistance, and obesity. If so, FIB4 index may reflect a coincidental onset of liver fibrosis (NASH and LC) [29] and CKD/DKD. Combined, we need to be careful to interpret whether the link between FIB4 index, including age, AST to ALT ratio and thrombocytopenia, and onset of CKD/DKD is a causal or a mere correlation relationship.

Second, an increase in the FIB4 index may be causally related to the onset of CKD/DKD. It is hypothesized that the progression of NAFLD into liver fibrosis (NASH) may be causally linked to the onset of CKD/DKD with four possible mechanisms: 1) A mechanism through the development of arteriosclerosis, in which NASH plays a crucial role by causing metabolic derangements such as dyslipidemia, insulin resistance, glucose intolerance, and dysproteinemia [33-36], and those concomitantly enhance the RAS system and deactivates nitric oxide synthesis [37,38]. The proatherosclerotic state in liver fibrosis can facilitate the development of CKD/DKD. 2) A mechanism mediated by liver-derived inflammatory mediators and oxidative stress: In NASH, activation of the inflammation and production of reactive oxygen species enhance the release of proinflammatory, procoagulant, pro-oxidant, and profibrinogenic factors from the liver and those hepatokines may be involved in the development of CKD/DKD [4,11,39,40]. 3) A mechanism through hepatorenal syndrome (HRS): HRS is usually regarded as a detrimental condition in patients with end stage liver failure, such as LC [41]. However, this condition might be involved, at least partly, in the development of CKD/DKD: CKD/DKD may be resulting from a decrease in renal blood flow caused by a decrease in effective circulating

blood volume due to whole body vasodilatation, increased portal pressure, and a decrease in cardiac output [41]. 4) A mechanism through insulin resistance (IR). Lipid accumulation in non-adipose tissues is called ectopic fat deposition [42], which typically occurs in the liver of individuals with visceral fat obesity. Increased fatty acid fluxes from visceral fat cause hepatic insulin resistance [43] which lead to simple hepatic steatosis. Vicious cycle of worsening insulin resistance in hepatic steatosis can promote the progression from simple fatty liver (NAFLD) to NASH possibly via a multifactorial process involving oxidative stress, lipid peroxidation, and mitochondrial dysfunction [44,45]. The net effect of hepatic insulin resistance elucidates insulin resistance in whole-body [44,45] and may also be linked to that in the kidney [46]. Thus, insulin resistance in the liver and kidney might contribute synergistically to the progression of kidney disease by various mechanisms, including worsening diabetic control, activation of the sympathetic nervous system, sodium retention, and downregulation of the natriuretic peptide system [8,46].

There are limitations in this study. First, since a liver biopsy was not performed, the correlation between the FIB4 index and the actual degree of fibrosis is not objective. Second, this was a retrospective cohort study and the causal or correlation relationship cannot be determined in this study. Third, this study comprised of only Japanese race from only two centers, suggesting a possibility of selection bias. Fourth, it could be arguable that respective assessment of “proteinuria” and “worsening eGFR” are clinically relevant or not [4]. Since progression of proteinuria is the main driver of the DKD, it might be meaningless to differentiate “proteinuria” and “worsening eGFR” separately.

Strength of this study. We performed several sensitivity analysis to validate our results. Cox proportional hazards models, by using continuous (Supplement Figure 2) and quartile variables (Supplement Figure 3), by time dependent covariate of follow-up HbA1c, and by multiple imputation method for missing data also found that the FIB4 index was a significant variable for the onset of DKD and proteinuria. Finally, time dependent ROC analysis of FIB4 index confirmed that this addition of FIB4 index may be useful during a longer period ~10 years.

Acknowledgment

Ethics approval and consent to participate

The study protocol was approved by the Fukushima Medical University Ethics Committee (Number 29118) and the Tomishiro Central Hospital Ethics Committee (R01R027). Written informed consent was obtained from the patients recruited between January 2018 and March 2019 in the Department of Diabetes, Endocrinology, and Metabolism, School of Medicine, Fukushima Medical University Hospital. Informed consent for participants in Tomishiro Central Hospital was waived by the Tomishiro Central Hospital Ethics Committee. Instead, we publicized information concerning this study in the Hospital and ensured that the subjects could refuse the use of their personal information.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing Interests

There was no involvement by the funding sources acknowledged in this study in any aspect of the study design, data collection, data analysis and interpretation, or writing of or decision to publish this manuscript.

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Authors' contributions

Study concept and design, analysis and interpretation of data, and drafting of the manuscript with input from all authors: H.S., M.S.; Acquisition of data: H.S., H.T., A.K., N.M., M.H., G.M., Technical supervision: Ka.A, A.T.; Administrative support: K.T., Ko.A., H.O., H.M, H.M., J.J.K., M.S. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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