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Fatty Acid-Binding Protein 4 in Patients with and without Diabetic Retinopathy

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Background: Fatty acid-binding protein 4 (FABP4) has been demonstrated to be a predictor of early diabetic nephropathy. However, little is known about the relationship between FABP4 and diabetic retinopathy (DR). This study explored the value of FABP4 as a biomarker of DR in patients with type 2 diabetes mellitus (T2DM).
Methods: A total of 238 subjects were enrolled, including 20 healthy controls and 218 T2DM patients. Serum FABP4 levels were measured using a sandwich enzyme-linked immunosorbent assay. The grade of DR was determined using fundus fluorescence angiography. Based on the international classification of DR, all T2DM patients were classified into the following three subgroups: non-DR group, non-proliferative diabetic retinopathy (NPDR) group, and proliferative diabetic retinopathy (PDR) group. Multivariate logistic regression analyses were employed to assess the correlation between FABP4 levels and DR severity.
Results: FABP4 correlated positively with DR severity (r=0.225, P=0.001). Receiver operating characteristic curve analysis was used to assess the diagnostic potential of FABP4 in identifying DR, with an area under the curve of 0.624 (37% sensitivity, 83.6% specificity) and an optimum cut-off value of 76.4 μg/L. Multivariate logistic regression model including FABP4 as a categorized binary variable using the cut-off value of 76.4 μg/L showed that the concentration of FABP4 above the cut-off value increased the risk of NPDR (odds ratio [OR], 3.231; 95% confidence interval [CI], 1.574 to 6.632; P=0.001) and PDR (OR, 3.689; 95% CI, 1.306 to 10.424; P=0.014).
Conclusion: FABP4 may be used as a serum biomarker for the diagnosis of DR.

Keywords: Biomarkers; Diabetes mellitus, type 2; Diabetic retinopathy; Diagnosis; Fatty acid-binding proteins

INTRODUCTION

Diabetic retinopathy (DR), a major microvascular complication of type 2 diabetes mellitus (T2DM), has aroused great public concern worldwide. The number of T2DM patients is expected to increase to 642 million by 2040 [1]. According to conservative estimates, approximately 60% of T2DM patients eventually develop DR in their lifetime [2]. A person with diabetes has a 25-fold increased risk of blindness compared to those without diabetes [3]. In particular, proliferative diabetic retinopathy (PDR) and diabetic macular edema (DME) have a strong propensity to cause vision impairments and even blindness. The number of patients with DR or vision-threatening diabetic retinopathy (VTDR) is growing rapidly, estimated to reach 190.0 million and 56.3 million by 2030, respectively [3]. Thus, DR is regarded as the main cause of adult blindness

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worldwide [4]. It is a well-acknowledged and evidence-based fact that DR shows few symptoms until retinal lesions are irreversible, posing great challenges to early identification and treatment of DR and placing a huge burden on public healthcare systems [5]. Clinically, less than half of patients have improved vision after injections of anti-vascular endothelial growth factor (VEGF), even though intraocular injections of anti-VEGF are the most effective therapy inhibiting the ocular neovascularization [6]. In addition, one or more complications have already occurred in approximately a quarter of patients with T2DM, even at first discovery [7]. Thus, early and timely identification of DR and new therapeutic strategies, except for inhibiting pathological angiogenesis, are urgently needed.

Fatty acid-binding protein 4 (FABP4) is abundantly expressed in adipocytes, capillary endothelial cells (ECs), and macrophages [8]. It exists in most bodily fluids [9]. The role of FABP4 was raised for the first time by Xu et al. [10], and numerous studies have demonstrated that FABP4 plays a crucial role in fatty acid delivery and utilization, cell proliferation and differentiation [11]. FABP4 has also been associated with obesity [10], metabolic syndrome [10], insulin resistance (IR) [12, 13], diabetes mellitus, atherosclerosis [14], and heart failure [13]. Moreover, previous studies have revealed a strong correlation between elevated serum FABP4 concentrations and a decline in kidney function in diabetic patients. Cabre et al. [15] first found that FABP4 correlated with estimated glomerular filtrate rate (eGFR) inversely and with serum creatinine (Scr) positively, but was not related to urinary albumin to creatinine ratio (UACR) in 2008. The same conclusion was drawn by Yeung et al. [16] a year later. A 5-year prospective study found that diabetic patients with higher FABP4 levels had a significantly increased risk of end-stage renal disease and other incident adverse renal outcomes [17]. Another study reported that FABP4 induces apoptosis in human mesangial cells via endoplasmic reticulum stress [18]. Given that DR is also a major microvascular complication, we hypothesized that there also exists a striking correlation between FABP4 and DR. Thus, the purpose of this study was to explore the value of FABP4 as a serum biomarker of DR.

METHODS

Study population

In the present study, 218 patients hospitalized for T2DM, whose median duration of diabetes was 8 years, were consecutively recruited between September 2017 and October 2018 at Affiliated Hospital of Nantong University. Diabetes mellitus was diagnosed based on the 1999 World Health Organization diagnostic criteria [19]. The exclusion criteria were as follows: type 1 diabetes mellitus, gestational diabetes mellitus, other special types of diabetes mellitus, acute complications of diabetes, kidney or retina damage caused by other factors, severe cardiopulmonary impairment, chronic liver diseases, chronic or acute inflammation, familial hypercholesterolemia, morbid obesity (body mass index [BMI] $\geq$40 kg/m^2), malignancy, or previous surgical or trauma history within the past 3 months. Twenty healthy individuals were randomly enrolled from the Medical Examination Center of Affiliated Hospital of Nantong University and comprised the control group. Each participant was informed of the purpose of the study, and all subjects provided written consent. The study was approved by the ethics committee of Affiliated Hospital of Nantong University (2018-k016) and was conducted in accordance with the principles of the Declaration of Helsinki.

Clinical measurements

The general clinical and anthropometric information of participants, such as age, sex, height, weight, waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), duration of diabetes, and past history were recorded at baseline. Dividing weight (kg) by the square of height (m^2) equals BMI (kg/m^2). Fasting blood samples were collected in the morning after admission, and the same samples were immediately centrifuged for serum FABP4 levels. Fasting plasma glucose (FPG), 2-hour postprandial plasma glucose (2hPG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), blood urea nitrogen (BUN), Scr, and serum uric acid (SUA) levels were determined using a fully automatic biochemical analyzer (Hitachi 7600-020, Hitachi, Tokyo, Japan). A modular analyzer (Cobas 8000, Roche Diagnostics GmbH, Mannheim, Germany) was used to measure serum cystatin C (Scys C). Another Roche Cobas e 411 analyzer was used to measure fasting insulin (FINS) and fasting C-peptide (FCP) (d-68305, Mannheim, Germany). Homeostasis model assessment-estimated insulin resistance (HOMA-IR) was calculated as follows: HOMA-IR=[FPG (mmol/L)×FINS (μU/mL)/22.5]. Glycosylated hemoglobin (HbA1c) levels were measured using high-performance liquid chromatography

(Bio-Rad Laboratories, Hercules, CA, USA). Immunonephelometry and a BN II analyzer were used to measure urinary creatinine and albumin concentrations (Siemens Diagnostics, Erlangen, Germany). UACR was calculated as the ratio of urinary albumin level to urinary creatinine level. GFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration creatinine–cystatin C equation (2012): $135 \times \min(Scr/\kappa, 1)^{\alpha} \times \max(Scr/\kappa, 1)^{-0.601} \times \min(Scys\ C/0.8, 1)^{-0.375} \times \max(Scys\ C/0.8, 1)^{-0.711} \times 0.995^{Age}$ ($\times 0.969$ if female) ($\times 1.08$ if black), where Scys C is serum cystatin C, κ is 0.7 for females and 0.9 for males, α is −0.248 for females and −0.207 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1 [20]. Serum FABP4 levels were measured using a sandwich enzyme-linked immunosorbent assay (BioVendor Laboratory Medicine, Modrice, Czech Republic). The lowest detection concentration for FABP4 was 0.39 μg/L. The grade of DR was determined using fundus fluorescence angiography. Based on the international classification of DR [21], all T2DM patients were classified into three subgroups: non-diabetic retinopathy (NDR) group, non-proliferative diabetic retinopathy (NPDR) group, and PDR group. Microaneurysms, cotton wool spots, hard exudates, intraretinal hemorrhages, venous beading changes, and intraretinal microvascular anomalies were categorized as NPDR. PDR referred to the presence of pathological neovascularization, vitreous or preretinal hemorrhage, as well as tractional retinal detachment. All other lesions were diagnosed as NDR.

Statistical analysis

Continuous statistics were presented as mean ± standard deviation. Data without a normal distribution were logarithmically transformed before analyses and were shown as median (interquartile range). If statistics were still non-parametrically distributed after transformation, they were analyzed using the Mann-Whitney *U* test or Kruskal-Wallis test. The chi-square test was used for categorical and qualitative variables, which were described as numbers. The comparison between two independent groups with normally distributed quantitative data was performed using an independent *t*-test. For three or more groups, one-way analysis of variance was used for comparison. Spearman correlation analysis was conducted to ascertain specific factors that may be related to serum FABP4 concentrations. Multivariate logistic regression analyses were performed to determine the independent influence of serum FABP4 levels on the severity of retinopathy, and were adjusted for age, sex, BMI, duration of diabetes, AST, HOMA-IR, and eGFR. Receiver operating characteristic (ROC) curve analysis was conducted to assess the diagnostic performance of FABP4 in identifying DR. All analyses were conducted using the SPSS statistical package version 26.0 (IBM Co., Armonk, NY, USA). Statistical significance was set at $P<0.05$.

RESULTS

Compared with the control group, T2DM patients were older ($P=0.018$) and heavier ($P=0.043$), and had a larger WC, higher SBP, FPG, 2hPG, HbA1c, HOMA-IR, TG, AST, ALP, BUN, and UACR ($P<0.05$), and lower Scr ($P=0.042$) and HDL ($P<0.001$) (Table 1). No statistical differences were observed in BMI, DBP, FINS, FCP, TC, LDL, ALT, SUA, Scys C, and eGFR. Interestingly, serum FABP4 levels in T2DM patients were significantly higher than those in the control group (T2DM group vs. the control group, 49.3 μg/L [interquartile range, IQR, 34.5 to 81.0] vs. 28.3 μg/L [IQR, 24.4 to 33.7]; $P<0.001$), which is consistent with prior studies [4].

Next, we focused on serum FABP4 concentrations during different periods of DR. On dividing all T2DM patients into three subgroups according to the international classification of DR, statistically significant differences were observed. As shown in Fig. 1A, serum FABP4 levels seemed to increase with the increasing severity of DR (NDR group vs. NPDR group vs. PDR group, 45.5 μg/L [IQR, 32.4 to 64.8] vs. 53.7 μg/L [IQR, 38.7 to 90.7] vs. 66.9 μg/L [IQR, 42.1 to 111.3]; $P=0.004$). Additionally, age ($P=0.011$), duration of diabetes ($P=0.004$), eGFR ($P=0.003$), and AST ($P=0.035$) were found to be significantly different among these three subgroups, suggesting that T2DM patients who were older, had a longer duration of diabetes, and worse renal and hepatic function tended to develop more severe DR (Table 1).

Spearman correlation analysis showed that serum FABP4 was positively correlated with age, female sex, BMI, FINS, and Scys C ($P<0.05$), and negatively correlated with eGFR ($r=-0.230$, $P=0.001$). A statistically significant correlation was found between FABP4 and HOMA-IR ($r=0.179$, $P=0.008$), which revealed that FABP4 increased with the presence of more severe IR (Table 2). In addition, this analysis showed a distinct association between increased FABP4 levels and the severity of DR ($r=0.225$, $P=0.001$) (Fig. 1B).

To examine the independent role of serum FABP4 in DR, multivariate logistic regression analysis involving the grade of

Table 1. Baseline characteristics of healthy controls and T2DM patients according to the international classification of diabetic retinopathy

Characteristic	Control (*n*=20)	T2DM (*n*=218)	NDR (*n*=110)	NPDR (*n*=84)	PDR (*n*=24)	*P* value[a]	*P* value[b]
Sex, male/female	11/9	136/82	68/42	57/27	11/13	0.515	0.143
Age, yr	49.0 (39.0–53.8)	56.0 (47.0–66.3)	54.09±14.28	56.79±13.11	63.04±9.72	0.018	0.011
Diabetes duration, yr	–	8.0 (2.0–12.0)	5.0 (0.9–10.0)	10.0 (5.0–10.0)	10.0 (7.0–13.5)	-	0.004
Age of diabetes diagnosis	–	48.0 (39.0–55.0)	48.0 (39.0–55.3)	47.0 (39.0–54.8)	53.0 (45.5–57.0)	-	0.220
Weight, kg	65.71±7.86	69.85±12.57	70.70±13.64	68.84±10.87	69.51±13.28	0.043	0.588
BMI, kg/m^2	23.5 (22.6–25.5)	24.6 (22.3–27.5)	25.1 (22.3–27.6)	23.9 (22.1–26.0)	25.7 (22.7–29.4)	0.246	0.132
WC, cm	73.70±6.01	90.01±11.57	90.0 (83.8–96.3)	89.0 (82.0–96.0)	90.0 (80.8–102.0)	<0.001	0.619
SBP, mm Hg	113.5 (109.3–130.0)	133.0 (121.8–145.3)	133.0 (122.8–142.8)	132.0 (119.0–144.0)	134.5 (123.8–153.0)	0.001	0.455
DBP, mm Hg	75.0 (64.3–79.5)	78.0 (71.0–85.0)	79.19±10.40	76.99±10.47	76.33±8.75	0.061	0.233
HbA1c, %	5.1 (4,7–5.4)	9.6 (8.0–11.1)	9.0 (7.9–11.0)	9.8 (8.4–11.1)	9.4 (7.3–12.1)	<0.001	0.416
FPG, mmol/L	5.1 (4.9–5.3)	10.0 (8.2–12.2)	10.0 (8.0–12.4)	9.9 (8.4–12.0)	10.1 (9.0–13.4)	<0.001	0.654
2hPG, mmol/L	7.10±0.50	21.57±5.27	21.7 (18.0–24.9)	21.5 (18.3–26.1)	22.7 (20.3–24.9)	<0.001	0.745
FINS, mIU/L	8.5 (5.3–13.6)	7.4 (3.7–13.3)	8.1 (4.0–14.1)	6.5 (3.4–11.7)	6.5 (4.4–26.0)	0.263	0.255
FCP, μg/L	1.9 (1.1–3.1)	1.9 (1.2–2.8)	2.1 (1.3–3.1)	1.8 (1.0–2.3)	1.8 (1.3–2.8)	0.993	0.090
HOMA-IR	1.9 (1.2–3.1)	2.9 (1.6–6.5)	3.7 (1.6–7.4)	2.7 (1.5–5.4)	2.7 (1.7–11.6)	0.021	0.223
TC, mmol/L	5.2 (4.3–5.4)	4.5 (3.7–5.5)	4.4 (3.7–5.6)	4.6 (3.8–5.6)	4.4 (3.6–5.0)	0.053	0.695
TG, mmol/L	1.0 (0.7–1.4)	1.5 (1.0–2.4)	1.6 (1.0–2.6)	1.4 (0.9–2.2)	1.5 (0.9–1.8)	0.011	0.172
HDL, mmol/L	1.4 (1.2–1.7)	1.1 (0.9–1.3)	1.0 (0.9–1.2)	1.1 (0.9–1.3)	1.1 (1.0–1.4)	<0.001	0.149
LDL, mmol/L	2.68±0.69	2.67±0.93	2.69±1.01	2.69±0.86	2.57±0.76	0.994	0.831
ALT, U/L	20.0 (18.0–22.0)	22.0 (16.0–34.0)	23.0 (16.0–37.3)	22.0 (14.0–31.8)	24.5 (15.3–35.3)	0.126	0.379
AST, U/L	16.5 (14.3–23.5)	20.0 (16.0–26.0)	20.5 (17.0–28.0)	19.0 (15.0–24.0)	23.0 (18.0–29.3)	0.037	0.035
ALP, U/L	70.0 (56.0–87.0)	84.5 (71.0–105.3)	83.0 (69.0–105.0)	84.5 (73.3–106.3)	87.0 (69.0–115.0)	0.003	0.610
BUN, mmol/L	4.7 (4.1–5.4)	5.4 (4.2–6.5)	5.30 (3.9–6.6)	5.6 (4.5–6.4)	5.3 (4.5–6.8)	0.049	0.287
SUA, μmol/L	306.5 (243.8–368.3)	283.0 (225.8–344.3)	283.0 (221.3–344.3)	286.5 (234.8–347.5)	271.0 (226.3–345.8)	0.219	0.794
Scr, mg/dL	0.7 (0.6–0.9)	0.7 (0.5–0.8)	0.6 (0.5–0.7)	0.7 (0.6–0.7)	0.7 (0.5–0.9)	0.042	0.305
UACR, mg/g	10.2 (3.5–13.2)	22.9 (8.8–81.5)	22.3 (7.8–64.9)	19.1 (8.0–106.1)	35.6 (10.8–84.0)	<0.001	0.445
Scys C, mg/L	0.7 (0.6–0.9)	0.6 (0.5–0.8)	0.6 (0.5–0.7)	0.6 (0.5–0.8)	0.7 (0.6–1.0)	0.299	0.083
eGFR, mL/min/1.73 m^2	115.7 (94.5–123.2)	116.2 (99.7–130.0)	118.19±24.82	112.70±27.02	97.86±29.28	0.485	0.003
FABP4, μg/L	28.3 (24.4–33.7)	49.3 (34.5–81.0)	45.5 (32.4–64.8)	53.7 (38.7–90.7)	66.9 (42.1–111.3)	<0.001	0.004

Values are presented as median (interquartile range) or mean±standard deviation.
T2DM, type 2 diabetes mellitus; NDR, non-diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin; FPG, fasting plasma glucose; 2hPG, 2-hour postprandial plasma glucose; FINS, fasting insulin; FCP, fasting C-peptide; HOMA-IR, homeostasis model assessment-estimated insulin resistance; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, lkaline phosphatase; BUN, blood urea nitrogen; SUA, serum uric acid; Scr, serum creatinine; UACR, urinary albumin to creatinine ratio; Scys C, serum cystatin C; eGFR, estimated glomerular filtration rate; FABP4, fatty acid-binding protein 4.
[a]*P* value for comparison between healthy controls and T2DM patients, [b]*P* value for comparison among the three diabetic subgroups.

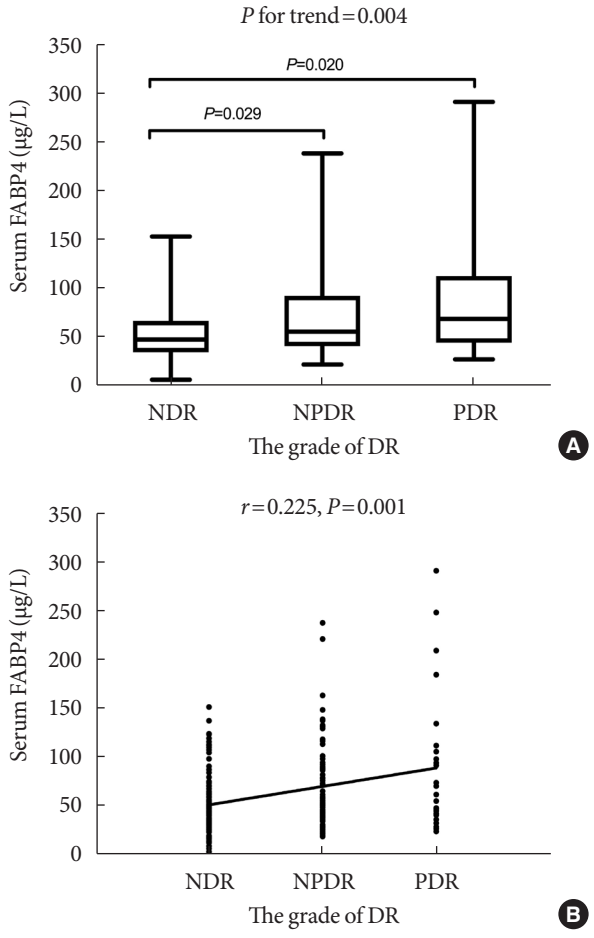

Fig. 1. (A) Comparison of serum fatty acid-binding protein 4 (FABP4) levels among subgroups divided by the severity of diabetic retinopathy (DR). *P* for trend by Kruskal-Wallis test. (B) The correlation between serum FABP4 levels and the severity of DR. NDR, non-diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

DR as a dependent variable was performed. As illustrated in Table 3, there was a statistically positive correlation between serum FABP4 concentrations and the severity of DR, even after adjustment for age, sex, BMI, duration of diabetes, AST, HOMA-IR, and eGFR ($P<0.01$). Taking the NDR group as the reference category, serum FABP4 was found to be a risk factor for NPDR (odds ratio [OR], 1.013; 95% confidence interval [CI], 1.005 to 1.022; $P=0.003$) and PDR (OR, 1.019; 95% CI, 1.008 to 1.030; $P=0.001$) in T2DM patients.

We then plotted the levels of FABP4 as a ROC curve to assess its diagnostic performance in identifying DR. According to the ROC curve shown in Fig. 2, as an indicator for the diagnosis of DR, the optimal cutoff value was 76.4 μg/L, the sensitivity and

Table 2. Correlation analysis for FABP4 in T2DM patients

Variable	FABP4[a]	
	r	*P* value
Female sex	0.168	0.013
Age, yr	0.140	0.039
Diabetes duration, yr	0.099	0.147
Age of diabetes diagnosis, yr	0.094	0.169
Weight, kg	0.110	0.104
BMI, kg/m^2	0.180	0.008
WC, cm	0.103	0.130
SBP, mm Hg	0.064	0.344
DBP, mm Hg	–0.070	0.306
HbA1c, %	0.032	0.638
FPG, mmol/L	0.111	0.102
2hPG, mmol/L	0.033	0.631
FINS, mIU/L	0.151	0.026
FCP, μg/L	0.046	0.501
HOMA-IR	0.179	0.008
TC, mmol/L	–0.059	0.385
TG, mmol/L	0.103	0.129
HDL, mmol/L	0.004	0.958
LDL, mmol/L	–0.108	0.111
ALT, U/L	0.004	0.954
AST, U/L	0.068	0.314
ALP, U/L	0.040	0.552
BUN, mmol/L	0.028	0.679
SUA, μmol/L	0.091	0.183
Scr, mg/dL	–0.005	0.939
UACR, mg/g	0.130	0.055
Scys C, mg/L	0.217	0.001
eGFR, mL/min/1.73 m^2	–0.230	0.001
The severity of DR	0.225	0.001

FABP4, fatty acid-binding protein 4; T2DM, type 2 diabetes mellitus; BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin; FPG, fasting plasma glucose; 2hPG, 2-hour postprandial plasma glucose; FINS, fasting insulin; FCP, fasting C-peptide; HOMA-IR, homeostasis model assessment-estimated insulin resistance; TC, total cholesterol; TG, triglyceride; HDL, high-density lipoprotein; LDL, low-density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; BUN, blood urea nitrogen; SUA, serum uric acid; Scr, serum creatinine; UACR, urinary albumin to creatinine ratio; Scys C, serum cystatin C; eGFR, estimated glomerular filtration rate; DR, diabetic retinopathy.
[a]Spearman correlation analysis for non-normally distributed variables.

Table 3. Multivariate logistic regression model for FABP4 as a continuous independent variable with the grade of diabetic retinopathy as a dependent variable

Variable	FABP4	
	OR (95% CI)	*P* value
NDR	Reference	
NPDR	1.013 (1.005–1.022)	0.003
PDR	1.019 (1.008–1.030)	0.001

Multivariate logistic regression model was adjusted for the following covariates: age, sex, body mass index, duration of diabetes, aspartate aminotransferase, homeostasis model assessment-estimated insulin resistance, and estimated glomerular filtration rate.
FABP4, fatty acid-binding protein 4; OR, odds ratio; CI, confidence interval; NDR, non-diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

Table 4. Multivariate logistic regression model for FABP4 as a categorical independent variable with the grade of diabetic retinopathy as a dependent variable

Variable	NPDR		PDR	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value
FABP4 ≤76.4 μg/L (*n*=160)	Reference	-	-	-
FABP4 >76.4 μg/L (*n*=58)	3.231 (1.574–6.632)	0.001	3.689 (1.306–10.424)	0.014

Reference category: The non-diabetic retinopathy group. Multivariate logistic regression analysis including FABP4 as a categorized binary variable using the cut-off value of 76.4 μg/L. FABP4 was given value 0 in patients with FABP >76.4 μg/L (*n*=58) and value 1 in patients with FABP ≤76.4 μg/L (*n*=160). This model was adjusted for the following covariates: age, sex, body mass index, duration of diabetes, aspartate aminotransferase, homeostasis model assessment-estimated insulin resistance, and estimated glomerular filtration rate.
FABP4, fatty acid-binding protein 4; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; OR, odds ratio; CI, confidence interval.

specificity were 37% and 83.6%, respectively, and the area under the curve was 0.624 (95% CI, 0.550 to 0.698).

To further investigate the correlation between FABP4 and the severity of DR, serum FABP4 concentrations were assessed as a binary variable using the cut-off value obtained from the ROC curve. It was incorporated in the multivariate logistic regression model as a categorical independent variable (with the lower FABP4 as reference) (Table 4). This increased the OR from 1.013 and 1.019, obtained using FABP4 as a continuous variable (Table 3), to 3.231 and 3.689, respectively. We found

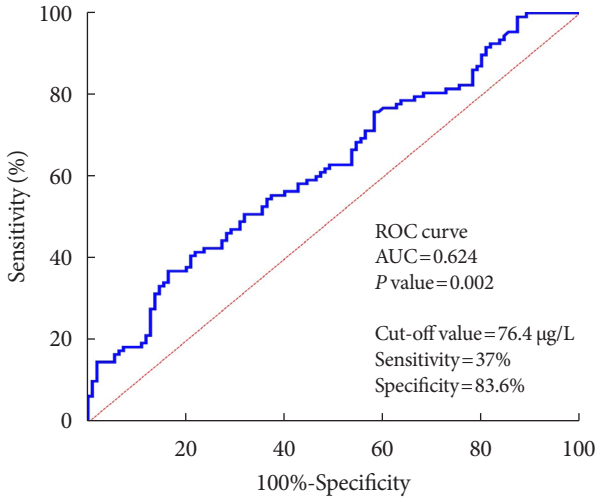

Fig. 2. Diagnostic performance of fatty acid-binding protein 4 in identifying diabetic retinopathy. ROC, receiver operating characteristic; AUC, area under curve.

that FABP4 concentrations above the cut-off value significantly increased the risk of NPDR (OR, 3.231; 95% CI, 1.574 to 6.632; *P*=0.001) and PDR (OR, 3.689; 95% CI, 1.306 to 10.424; *P*=0.014), suggesting the potential of serum FABP4 as a novel biomarker of DR.

DISCUSSION

Our retrospective study showed that serum FABP4 levels were independently and positively correlated with the severity of retinopathy. In addition, FABP4 had great potential as a vital risk factor for NPDR and PDR. A 5-year prospective study showed that diabetic patients with higher quartiles of FABP4 at admission had a tendency to develop severe DR, revealing a positive relationship between baseline serum FABP4 concentrations and the severity of DR [4]. Furthermore, this follow-up study ultimately revealed the role of FABP4 as an independent prognostic biomarker of DR or VTDR in diabetic patients. Interestingly, Itoh et al. [9] confirmed the existence of FABP4 in the vitreous fluid of PDR patients and found that the vitreous concentration of FABP4 was significantly higher in PDR patients than in non-PDR patients. These observations strongly suggest that FABP4 may play a partial role in the pathogenesis of DR.

The pathophysiology of DR is an extremely complex, multifactorial, and systematic process induced by long-term exposure to hyperglycemia [22]. Essentially, it is an ischemic dis-

ease. Microvascular abnormalities (loss of pericytes, dysfunction and proliferation of ECs, and thickening of the basement membrane) lead to changes in retinal local hemodynamics and hypoxia [23,24]. Thus, multiple cytokines, chemokines, and growth factors are released under hypoxia, leading to low-grade inflammation, first local, then chronic and systemic [25]. For instance, intercellular adhesion molecule-1 causes the accumulation of leukocytes around the vascular walls of retinal capillaries, disrupting the tight junctions between ECs, increasing vascular permeability, and breaking down the blood retinal barrier (BRB) [26,27]. Interestingly, VEGF-A and tumor necrosis factor α (TNF-α) have been shown to induce vascular permeability and break down BRB by activating atypical protein kinase C (aPKC) [28]. Additionally, hypoxia-inducible factor 1 is overexpressed in the presence of high glucose [29]. It then highly induces the expression and accumulation of VEGF-A, the most powerful factor in stimulating angiogenesis, thus turning on the angiogenic switch and leading to active pathological angiogenesis [30]. Thus, pathological neovascularization and chronic low-grade inflammation induced by hyperglycemia are the main characteristic features of DR [31].

The precise mechanisms underlying the elevation of FABP4 and its possible role in the pathogenesis of DR remain unclear. However, several plausible explanations have been suggested. FABP4 may promote EC proliferation and angiogenesis [8]. VEGF-A, which is a crucial mediator of pathological neovascularization in oxygen-induced retinopathy (OIR), induces the expression of FABP4 in ECs [8]. FABP4-deficient ECs increased the expression of cleaved caspase 3 and exhibited impaired migration and invasion; the former is an apoptotic marker, and the latter plays a key role in angiogenesis. Similarly, VEGF-A-induced sprouting in an *in vitro* model of angiogenesis, such as the aortic ring model, was inhibited by FABP4 suppression [32]. In addition, Saint-Geniez et al. [33] established a mouse model of OIR. They observed that the expression of FABP4 mRNA was obviously upregulated in the retina, especially when the pathological angiogenic response was the most active. The amount of pathological neovascularization was significantly reduced in FABP4 knockout mice compared to that in wild-type controls. All loss-of-function analyses *in vivo* and *in vitro* revealed that FABP4 in ECs mediates VEGF-A-dependent proliferative and pro-angiogenic effects. Furthermore, FABP4 promotes angiogenesis mainly through the stem cell factor/c-kit pathway [32]. Thus, we believe that FABP4 may induce pathological neovascularization in the retina.

Intriguingly, circulating serum FABP4 levels were also confirmed to be inversely associated with endothelial function in T2DM patients, according to a study by Aragones et al. [34]. Additionally, FABP4 may increase the production of nitric oxide and decrease the activation of endothelial nitric oxide synthase by inhibiting the insulin-signaling pathway [35], leading to endothelial dysfunction. During the progression of DR, hyperglycemia also induces oxidative stress [36]; the latter induces the expression of FABP4 in ECs [37].

FABP4 may also exhibit pro-inflammatory properties. Previous internal and external studies have reported that inflammation in adipocytes mediated via the p38/nuclear factor-kappa B signaling pathway can be induced by exogenous FABP4 (eFABP4) [38]. As a novel monoclonal antibody against FABP4, 2E4 can significantly decrease the mRNA and protein levels of FABP4 in the adipose tissue of mice [39]. The suppression of FABP4 expression downregulated the expression of pro-inflammatory cytokines in adipose tissue, including monocyte chemotactic protein-1, TNF-α, and interleukin-6 [39]. Clinically, it is much more effective to treat DME with a combination of anti-VEGF and steroids, rather than anti-VEGF alone, which validates the vital role of inflammation in the progression of DR.

Parvanova et al. [40] found that more severe IR is associated with PDR. As pointed out previously, we detected a strong and positive association between persistently augmented FABP4 and IR, which is not surprising since many large cross-sectional and follow-up studies have already uncovered this phenomenon [12,13]. In addition, FABP4 can be regarded as an independent biomarker of IR [41], and can be employed to predict the development of IR in recipients of liver transplants [42]. Consistent with these observations, glucose homeostasis and insulin sensitivity in genetically obese mice can be significantly improved by treatment with FABP4 inhibitors, such as BMS309403 [14]. The same effect can be achieved by knocking out the FABP4 gene [43]. Accumulation of ectopic lipids due to decreased utilization of fatty acids has been proven to be a key factor in IR [44]. As mentioned earlier, FABP4 is a lipid chaperone that plays a crucial role in fatty acid delivery and utilization [11]. FABP4 has also been shown to act like glucagon-like peptide-1, stimulating islet beta cells and altering insulin secretion [45].

Our study had several limitations. As a single-center, retrospective study, the causality between elevated FABP4 levels and

DR could not be determined. Moreover, the sample size of PDR was relatively small; thus, it may have affected the significant differences in the results. Therefore, follow-up studies on a larger scale should be conducted in multiple centers to further verify the results. Further molecular studies are needed to investigate the mechanism underlying the elevation of FABP4 in diabetic patients with DR.

In conclusion, circulating serum FABP4 concentrations were positively correlated with the severity of DR. In addition, this study proved that higher concentrations of FABP4 can cause a substantial increase in the risk of DR in T2DM patients. Therefore, FABP4 may be a potential biomarker for the diagnosis of DR.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

AUTHOR CONTRIBUTIONS

Conception or design: P.H., X.Z., Z.T., Y.G.
Acquisition, analysis, or interpretation of data: P.H., X.Z., Z.T., Y.G.
Drafting the work or revising: P.H., X.Z., Y.S., X.W., R.O., Y.J., X.Z., R.H., Z.T., Y.G.
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