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# Association of plasma galectin-3 and fetuin-A levels with diabetic retinopathy in type 2 diabetes mellitus patients

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## Abstract

**Introduction:** Galectin-3 (Gal-3) and fetuin-A (Fet-A) are cytokines that participate in inflammation and insulin resistance. Previous studies have found that altered Gal-3 and Fet-A levels in circulation correlate with diabetic complications. However, whether they are all associated with diabetic retinopathy (DR) has been little investigated. The aim of this study was to assess plasma Gal-3 and Fet-A concentrations, and to investigate their associations with the presence of DR in type 2 diabetes mellitus (T2DM) patients.

**Material and methods:** A total of 100 T2DM patients were enrolled, among which there were 50 patients without DR (non-diabetic retinopathy, NDR group) and 50 patients with DR (DR group). Clinical parameters were collected, and plasma Gal-3 and Fet-A levels were measured by enzyme-linked immunosorbent assay (ELISA).

**Results:** Both Gal-3 and Fet-A were found to be increased in DR patients with respect to NDR controls, and Gal-3 correlated positively with Fet-A. Bivariate correlation analysis revealed that Gal-3 levels were positively correlated with haemoglobin $A_{1c}$ ($HbA_{1c}$), while Fet-A correlated negatively with fasting C peptide (FC-P) and positively with homocysteine (Hcy). Binary logistic regression suggested that elevated Gal-3 and Fet-A levels were related to increased risk of DR. ROC curve displayed that the combination of Fet-A and Gal-3 exhibited better diagnostic value for DR.

**Conclusions:** Both Gal-3 and Fet-A were elevated in the circulation of DR patients, and they were positively associated with the occurrence of DR. The combination of 2 indicators showed better diagnostic value for DR. **(Endokrynol Pol 2023; 74 (5): 536–543)**

**Key words:** *type 2 diabetes mellitus; diabetic retinopathy; galectin-3; fetuin-A*

## Introduction

Diabetes mellitus (DM) constitutes an unrelenting global epidemic affecting more than 422 million people worldwide, and its prevalence is expected to increase to 592 million by 2035 [1]. Diabetic retinopathy (DR) is one of the most common severe microvasculature complications of DM and remains a leading cause of vision loss and blindness in working adults [2]. Because DR is generally asymptomatic and undetectable in the early stages, it is of significant importance to find circulating biomarkers for early prediction or diagnosis.

Galectin-3 (Gal-3), a member of an evolutionarily conserved family of soluble $\beta$-galactosidase-binding lectins [3], has been found expressed in multiple cell types including immune cells, epithelial cells, endothelial cells, and sensory neurons [4]. Evidence indicates that Gal-3 overexpression in retina tissue [5, 6] and RPE cells [4] participates in the pathogenesis of DR. Gal-3 can be detected in circulation, and its level was found to be positively correlated with obesity, insulin resistance, DM, diabetic macrovascular complications, and diabetic nephropathy [7–11]. However, data about circulating Gal-3 levels and DR are limited.

Fetuin-A (Fet-A) is a 64-kDa heterodimeric glycoprotein produced by the adipose tissue and liver [12]. Evidence indicates that circulating Fet-A is positively associated with insulin resistance, obesity, and T2DM [3]. Moreover, Fet-A was also found to stimulate pro-angiogenic factors, such as vascular endothelial growth factor (VEGF) [13–15]. Thus, the Fet-A level could be an early initiator of DR [13]. However, the cor-

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relation of circulating Fet-A level with DR has been little checked.

Therefore, the aim of the present study was to examine circulating levels of Gal-3 and Fet-A in T2DM patients with or without DR, and to check whether they can be markers for early diagnosis of DR.

## Material and methods

### *Patient enrollment*

This study was conducted in the Department of Endocrinology of the Third Hospital of Hebei Medical University from April 2021 to October 2021. There were a total of 100 T2DM patients of age range 30–75 years, enrolled according to the inclusion and exclusion criteria. Inclusion criteria: T2DM diagnosis according to World Health Organization (WHO, 1999). Exclusion criteria: T2DM patients during pregnancy, presenting with diabetes-related acute complications, such as diabetic ketoacidosis, hyperglycaemic hyperosmolar state, and lactic acidosis, or having comorbidities such as infectious diseases, autoimmune diseases, malignancy, heart failure, and renal and hepatic functional impairment.

DR was diagnosed by a professional ophthalmologist according to the Early Treatment of Diabetic Retinopathy Study (ETDRS) scale [16]. A detailed ophthalmologic examination was performed, and slit lamp biomicroscopy and retinopathy status were evaluated by fundus photography, fluorescein angiography, and optical coherence tomography.

This study was proved by the Ethics Committee of the Third Hospital of Hebei Medical University (No. W2021-084-1), and informed consent forms were signed by all patients.

### *Clinical data and blood sample collection*

Baseline data including age, gender, height, weight, body mass index (BMI), duration of diabetes, family history, and history of smoking and drinking alcohol of the patients were collected.

In total, 5 ml of fasting blood was collected from each patient. Levels of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein cholesterol (VLDL-C), uric acid (UA), creatinine (Cr), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), C-reactive protein (CRP), homocysteine (Hcy), fasting C peptide (FC-P), and haemoglobin $A_{1c}$ ($HbA_{1c}$) were measured in the biochemical laboratory. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation.

The plasma levels of Gal-3 and Fet-A were measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Wuhan Gorgeous Creatures) according to the protocol.

### *Statistical analysis*

All statistical analyses were performed using SPSS software (version 24), and graphs were drawn using GraphPad Prism 8.0 software. Normality of continuous data was examined by the Shapiro-Wilk test. Normally distributed parameters were expressed as mean ± standard deviation (mean ± SD) while non-normally distributed data were expressed as median and inter-quartile range (IQR). Independent sample t-test or Mann-Whitney U tests were performed to check the differences between two groups. Categorical variables were expressed as numbers and compared using the chi-square test. Spearman's rank correlation test and Pearson correlation analysis were performed to evaluate the correlations between the study factors and the clinical and biochemical parameters. Binary logistic regression was performed to detect the contribution of study parameters to predicting the onset of DR. Receiver operating characteristic (ROC) curve analysis was done to evaluate the diagnostic value of parameters for DR. Two-sided p-values $< 0.05$ were considered statistically significant.

## Results

### *Demographic, clinical, and biochemical characteristics of the study subjects*

In the present study, there were no significant differences observed in patients' baseline characteristics including gender, age, BMI, family history, and histories of smoking and drinking alcohol between NDR and DR patients (p all $> 0.05$). Compared to NDR patients, the duration of diabetes was longer in patients with DR ($p = 0.016$).

Compared with NDR patients, circulating levels of 25(OH)D and FC-P were significantly lower in DR patients (p all $< 0.05$). There were no differences found in other biochemical parameters including $HbA_{1c}$, TC, TG, LDL-C, VLDL-C, HDL-C, UA, Cr, eGFR, ALB, ALT, AST, Hcy, and CRP between DR and NDR patients (p all $> 0.05$) (Tab. 1).

### *Plasma Gal-3 and Fet-A levels*

In the present study, compared to patients in the NDR group, both Gal-3 and Fet-A levels were significantly increased in DR patients (p all $< 0.05$, Fig. 1AB), indicating that DR patients have increased circulating Gal-3 and Fet-A levels.

### *Correlation analysis of Gal-3, Fet-A, and 25(OH)D*

Bivariate correlation analysis was performed for plasma Gal-3 and Fet-A to check their relevant factors. Gal-3 was positively correlated with Fet-A level ($r = 0.623$, $p < 0.001$) and $HbA_{1c}$ ($r = 0.207$, $p = 0.041$). However, Gal-3 levels were not associated with age, TC, TG, HDL-C, LDL-c, eGFR, or other variables. Data also showed that Fet-A was negatively correlated with FC-P ($r = –0.248$, $p = 0.016$) but positively with Hcy ($r = 0.224$, $p = 0.026$) (Tab. 2).

Pearson correlation analysis was performed to check the correlation between serum levels of 25(OH)D and clinical parameters. Nevertheless, there were no correlations found between 25(OH)D level and other biochemical parameters including glucose, lipid profile, UA, Cr, Hcy, and CRP (Tab. 2).

### *Association of presence of DR with plasma Gal-3 and Fet-A levels*

From quartile 1 to quartile 4 of Gal-3, the percentage of DR was 12%, 20%, 30%, and 38%, respectively. From quartile 1 to quartile 4 of Fet-A, the percentage of DR was 12%, 18%, 28%, and 42%, respectively. Therefore, with increasing plasma Gal-3 and Fet-A levels, the per-

**Table 1.** *Demographic, clinical, and biochemical characteristics among study subjects*

| Characteristic | NDR | DR | p-value |
|---|---|---|---|
| Number | 50 | 50 | |
| Age [year] | 54.12 ± 11.23 | 56.88 ± 10.46 | 0.207 |
| Gender (female/male) | 16/34 | 22/28 | 0.303 |
| Duration [years] | 7.71 ± 6.86 | 10.91 ± 6.17 | 0.016* |
| BMI [kg/m$^2$] | 25.83 ± 2.81 | 26.10 ± 2.73 | 0.626 |
| $HbA_{1c}$ (%) | 8.67 ± 2.33 | 9.21 ± 1.80 | 0.200 |
| Family history (yes/no) | 26/24 | 29/21 | 0.688 |
| Smoking habit (yes/no) | 20/30 | 17/33 | 0.679 |
| Drinking alcohol (yes/no) | 15/35 | 18/32 | 0.671 |
| TC [mmol/L] | 5.19 ± 1.31 | 4.85 ± 1.24 | 0.191 |
| TG [mmol/L] | 2.28 ± 1.51 | 1.86 ± 1.14 | 0.122 |
| LDL-C [mmol/L] | 3.15 ± 0.84 | 2.88 ± 0.89 | 0.113 |
| HDL-C [mmol/L] | 1.20 ± 0.27 | 1.19 ± 0.25 | 0.859 |
| VLDL-C [mmol/L] | 1.01 ± 0.66 | 0.85 ± 0.51 | 0.170 |
| ALB [g/L] | 44.79 ± 3.86 | 43.64 ± 3.97 | 0.146 |
| ALT [U/L] | 24.74 ± 14.30 | 24.00 ± 13.21 | 0.789 |
| AST [U/L] | 20.70 ± 8.65 | 20.94 ± 8.99 | 0.892 |
| Cr [umol/L] | 70.75 ± 26.42 | 73.35 ± 31.50 | 0.656 |
| eGFR [mL/min/1.73 m$^2$] | 96.37 ± 18.35 | 91.76 ± 23.12 | 0.272 |
| UA [umol/L] | 350.36 ± 111.43 | 316.32 ± 93.89 | 0.102 |
| CRP [mg/L] | 2.55 ± 1.77 | 2.66 ± 2.49 | 0.848 |
| FC-P [ng/mL] | 2.86 ± 1.38 | 2.21 ± 1.12 | 0.014* |
| Hcy [umol/L] | 15.65 ± 5.65 | 16.06 ± 14.19 | 0.850 |
| 25(OH)D [ng/mL] | 17.96 ± 4.47 | 16.19 ± 3.78 | 0.043* |

Data presented as mean ± standard deviation or median (inter-quartile range). DR — diabetes retinopathy; BMI — body mass index; $HbA_{1c}$ — haemoglobin $A_{1c}$; TC — total cholesterol; TG — triglycerides; HDL-C — high-density lipoprotein cholesterol; LDL-C — low-density lipoprotein cholesterol; VLDL-C — very low-density lipoprotein cholesterol; ALB — albumin; ALT — alanine aminotransferase; AST — aspartate aminotransferase; Cr — creatinine; eGFR — estimated glomerular filtration rate; UA — uric acid; CRP — C-reactive protein; FC-P — fasting C peptide; Hcy — homocysteine; 25(OH)D — 25-hydroxyvitamin D. *Statistically significant ($p < 0.05$)

centage of DR presented an overall upward trend ($p < 0.05$).

Binary logistic regression analysis indicated that Gal-3 and Fet-A levels were positively associated with DR after adjusting for confounding variables including age, sex, duration of diabetes, BMI, $HbA_{1c}$, LDL-c, and eGFR ($p < 0.05$) (Tab. 3). In addition, binary logistic regression analysis performed with the presence of DR as a dependent variable and Gal-3 and Fet-A quartiles as independent variables. The fourth quartile of Fet-A showed a significantly increased odds ratio of 15.92 [95% confidence interval (CI): 2.55–99.47; $p = 0.003$] for DR with respect to its first quartile value. Third quartiles of Gal-3 showed a significantly increased odds ratio of 10.23 (95% CI: 1.74–60.18; $p = 0.01$) for DR compared with the bottom quartile after adjusting for confounding variables (Tab. 4).

### *ROC curve analysis*

ROC curve analysis was performed to check the diagnostic value of Gal-3 and Fet-A for DR. The area under the curve (AUC) for Fet-A in the diagnosis of DR was 0.754 (95% CI: 0.658–0.850; $p < 0.001$), and at a cut-off point set at 168.13 ng/mL, the sensitivity was 78% and specificity was 66%. The AUC for Gal-3 was 0.745 (95% CI: 0.650–0.840; $p < 0.001$), and at a cut-off point set at 5.39 ng/ml, the sensitivity was 70% and specificity was 68%. In addition, the AUC of Gal-3 and Fet-A combination was 0.793 (95% CI: 0.704–0.882; $p < 0.001$), indicating that the combination of these 2 factors had better diagnostic value for DR (Fig. 2).

## Discussion

Diabetic retinopathy is a severe microvascular complication that causes a heavy socioeconomic burden

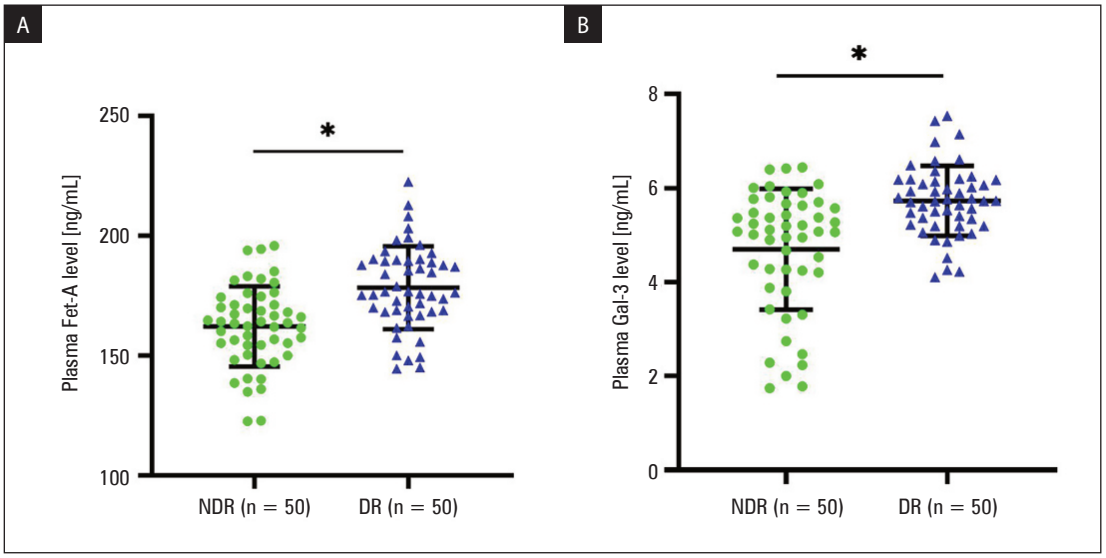


**Figure 1.** *Circulating concentrations of fetuin-A (Fet-A) (***A***) galectin-3 (Gal-3) (***B***) and in the non-diabetic retinopathy (NDR) and diabetic retinopathy (DR) groups. *Statistically significant (p < 0.05)*

**Table 2.** *Correlation analysis of circulating galectin-3 (Gal-3), 25-hydroxyvitamin D [25(OH)D], and fetuin-A (Fet-A)*

| | Gal-3 | | 25(OH)D | | Fet-A | |
|---|---|---|---|---|---|---|
| | r | p | r | p | r | p |
| Gal-3 | 1 | | –0.046 | 0.659 | 0.623 | < 0.001* |
| 25(OH)D | –0.046 | 0.659 | 1 | | –0.051 | 0.624 |
| Fet-A | 0.623 | < 0.001* | –0.051 | 0.624 | 1 | |
| Age | 0.065 | 0.519 | –0.129 | 0.217 | 0.020 | 0.840 |
| Duration | 0.157 | 0.119 | –0.048 | 0.645 | 0.051 | 0.611 |
| BMI | 0.036 | 0.724 | –0.096 | 0.362 | 0.015 | 0.879 |
| $HbA_{1c}$(%) | 0.207 | 0.041* | –0.013 | 0.902 | 0.184 | 0.07 |
| CRP | 0.051 | 0.703 | –0.236 | 0.075 | 0.127 | 0.344 |
| TC | –0.080 | 0.429 | –0.162 | 0.119 | –0.147 | 0.143 |
| TG | –0.016 | 0.874 | –0.088 | 0.400 | –0.119 | 0.239 |
| HDL-C | –0.028 | 0.782 | –0.070 | 0.502 | –0.099 | 0.325 |
| LDL-C | –0.083 | 0.413 | –0.172 | 0.097 | –0.156 | 0.121 |
| VLDL-C | –0.036 | 0.723 | –0.084 | 0.422 | –0.119 | 0.237 |
| UA | 0.036 | 0.725 | 0.078 | 0.452 | 0.087 | 0.390 |
| eGFR | –0.136 | 0.178 | 0.172 | 0.098 | –0.044 | 0.663 |
| FC–P | –0.146 | 0.163 | –0.053 | 0.627 | –0.248 | 0.016* |
| Hcy | 0.141 | 0.163 | –0.026 | 0.806 | 0.224 | 0.026* |

Data presented as correlation coefficient (r). DR — diabetes retinopathy; BMI — body mass index; $HbA_{1c}$ — haemoglobin $A_{1c}$; TC — total cholesterol; TG — triglycerides; HDL-C — high-density lipoprotein cholesterol; LDL-C — low-density lipoprotein cholesterol; VLDL-C — very low-density lipoprotein cholesterol; ALB — albumin; ALT — alanine aminotransferase; AST — aspartate aminotransferase; Cr — creatinine; eGFR — estimated glomerular filtration rate; UA — uric acid; CRP — C-reactive protein; FC-P — fasting C peptide; Hcy — homocysteine; 25(OH)D — 25-hydroxyvitamin D. *Statistically significant (p < 0.05)

worldwide [17]. Although substantial improvements have been made in the treatment of DR, its prevalence continues with the increment of DM patients [18]. So far, the diagnostic method for DR has been very limited, and special circulating markers for DR are lacking.

Gal-3 is a carbohydrate-binding protein that plays important regulatory roles in inflammation, oxidative

**Table 3.** *Binary logistic regression analysis of the independent factors for the presence of diabetic retinopathy (DR) [galectin-3 (Gal-3) and fetuin-A (Fet-A) as continuous variables]*

| Model | OR | 95% CI | p-value |
|---|---|---|---|
| **1** | | | |
| Fet-A | 1.041 | 1.007–1.076 | 0.017* |
| Gal-3 | 2.405 | 1.278–4.524 | 0.007* |
| **2** | | | |
| Fet-A | 1.041 | 1.005–1.079 | 0.024* |
| Gal-3 | 2.401 | 1.250–4.610 | 0.009* |
| Duration (years) | 1.106 | 1.014–1.206 | 0.023* |
| Age | 0.999 | 0.944–1.057 | 0.970 |
| Sex | 0.391 | 0.118–1.293 | 0.124 |
| $HbA_{1c}$ | 1.091 | 0.862–1.381 | 0.470 |
| **3** | | | |
| Fet-A | 1.041 | 1.003–1.081 | 0.036* |
| Gal-3 | 2.495 | 1.245–5.001 | 0.010* |
| Duration (years) | 1.132 | 1.031–1.243 | 0.009* |
| Age | 1.022 | 0.956–1.093 | 0.522 |
| Sex | 0.337 | 0.091–1.249 | 0.104 |
| $HbA_{1c}$ | 1.123 | 0.872–1.445 | 0.369 |
| BMI | 1.139 | 0.919–1.411 | 0.235 |
| LDL-C | 0.566 | 0.273–1.174 | 0.126 |
| eGFR | 1.014 | 0.986–1.043 | 0.334 |

Model 1: not adjusted for any variable; Model 2: adjusted for age, sex, duration of diabetes, and $HbA_{1c}$; Model 3: adjusted for model 2, BMI, LDL-C and eGFR. OR: odds ratio; 95% CI: 95% confidence interval; $HbA_{1c}$ — haemoglobin $A_{1c}$; BMI — body mass index; LDL-C — low-density lipoprotein cholesterol; eGFR — estimated glomerular filtration rate; *Statistically significant ($p < 0.05$)

stress, apoptosis, and angiogenesis [19]. Evidence indicates that elevated Gal-3 expression participates in retinal tissue inflammation in diabetic animals [6] and RPE cell oxidative damage caused by high glucose [4]. Increased circulating levels of Gal-3 have been found to be positively correlated with T2DM and diabetic nephropathy [20]. In the present study, we found that plasma Gal-3 level was increased in DR patients and positively correlated with DR. Similar findings have been described by Kumar et al., who showed that increased circulating Gal-3 levels were correlated with the incidence and severity of DR [19].

Data about circulating Gal-3 and its influencing factors in diabetic patients have been largely studied in recent years, but the conclusions are quite inconsistent. It has been found that circulating Gal-3 levels are negatively correlated with HDL-C [21, 22], positively correlated with CRP, erythrocyte sedimentation rate (ESR), duration of diabetes, albuminuria [23, 24], and BMI [25], and negatively [25] or positively [19] correlated with $HbA_{1c}$. In the present study, we found that Gal-3 was only positively correlated with $HbA_{1c}$; the different study population might have caused the differences.

Fet-A is a multifunctional glycoprotein that processes complicated functions in regulating inflammation, glucose homeostasis, energy homeostasis, and adipocyte metabolism, which participate in the pathogenesis of T2DM and its complications [26, 27]. It has been reported that higher Fet-A levels correlate with increased risk of retinopathy in diabetic patients [12, 13]. Concordant with prior findings, in the present study we found

**Table 4.** *Binary logistic regression analysis of the independent factors for the presence of diabetic retinopathy (DR) [galectin-3 (Gal-3) and fetuin-A (Fet-A) as categorical variables]*

| Variable | Model 1 | | Model 2 | | Model 3 | |
|---|---|---|---|---|---|---|
| | OR (95% CI) | p-value | OR (95% CI) | p-value | OR (95% CI) | p-value |
| **Gal-3** | | | | | | |
| Gal-3 quartile 1 | Ref | | Ref | | Ref | |
| Gal-3 quartile 2 | 2.47 (0.60–10.14) | 0.210 | 2.68 (0.57–12.57) | 0.211 | 3.57 (0.64–19.92) | 0.147 |
| Gal-3 quartile 3 | 5.22 (1.21–22.48) | 0.026* | 7.17 (1.49–34.62) | 0.014* | 10.23 (1.74–60.18) | 0.010* |
| Gal-3 quartile 4 | 4.96 (1.14–21.52) | 0.033* | 4.35 (0.92–20.58) | 0.063 | 5.11 (0.97–26.85) | 0.054 |
| **Fet-A** | | | | | | |
| Fet-A quartile 1 | Ref | | Ref | | Ref | |
| Fet-A quartile 2 | 1.14 (0.29–4.38) | 0.854 | 1.45 (0.33–6.40) | 0.627 | 1.38 (0.29–6.56) | 0.688 |
| Fet-A quartile 3 | 2.04 (0.50–8.42) | 0.323 | 2.22 (0.49–9.99) | 0.300 | 1.67 (0.32–8.62) | 0.539 |
| Fet-A quartile 4 | 10.45 (2.31–47.27) | 0.002* | 12.15 (2.31–63.80) | 0.003* | 15.92 (2.55–99.47) | 0.003* |

Model 1: not adjusted for any variable; Model 2: adjusted for age, sex, duration of diabetes, and HbA1c; Model 3: adjusted for model 2, BMI, LDL-C, and eGFR. OR — odds ratio; 95% CI — 95% confidence interval; $HbA_1$ — haemoglobin $A_{1c}$; BMI — body mass index; LDL-C — low-density lipoprotein cholesterol; eGFR — estimated glomerular filtration rate; *Statistically significant ($p < 0.05$)

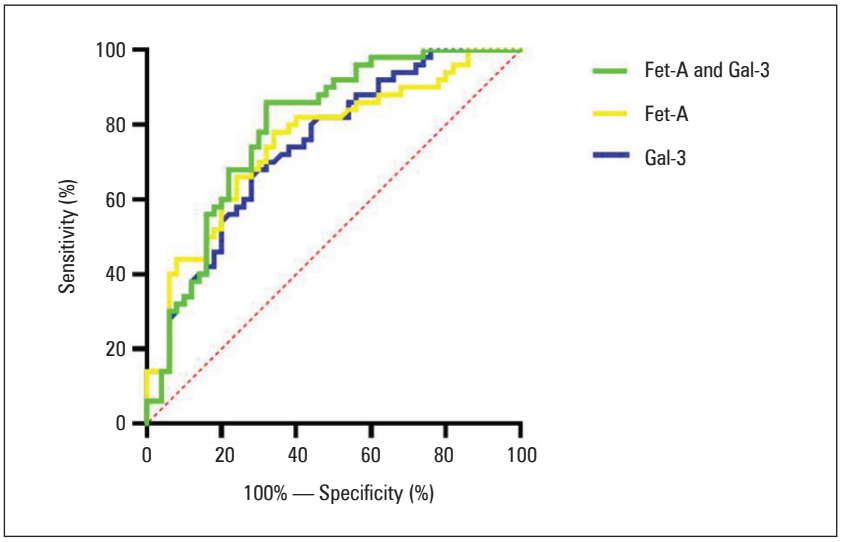


**Figure 2.** *Receiver operating characteristic (ROC) curve for galectin-3 (Gal-3) and fetuin-A (Fet-A) with respect to patients with diabetic retinopathy (DR) versus without retinopathy. The comparison of the area under the curve (AUC) was performed by a p-value < 0.05*



that elevated plasma Fet-A levels were associated with DR, which also has diagnostic value for DR.

Currently, the associated factors of circulating Fet-A remain uncertain because studies have shown different or even contradictory results. Studies conducted in prediabetic and diabetic populations found that Fet-A was positively correlated with BMI, waist circumference [28, 29], TG, and $HbA_{1c}$ [29, 30] and negatively or positively associated with HDL-C and CRP [29–32]. However, we failed to show any relationship between Fet-A and the above parameters. In the present study, we found that the Fet-A level was positively correlated with Hcy and negatively correlated with FC-P. More studies are needed to further clarify this issue.

Also in the present study, we found that the circulating Gal-3 level was positively correlated with Fet-A, which has never been reported before. Priya et al. [13] and Zhou et al. [14] reported that serum Fet-A levels were positively correlated with VEGF levels in DR patients. Meanwhile, it has been reported that Gal-3 may promote angiogenesis via upregulation of VEGF expression in the diabetic retina [5]. Therefore, there might be a relationship between Fet-A and Gal-3, but more studies on the matter are required. Moreover, the diagnostic value for DR was found in both Gal-3 and Fet-A, while the diagnostic value was better in the combination of these 2 factors, which might be prospective for DR screening.

Vitamin D receptors are expressed in the retina [33]. The correlation of circulating vitamin D with DR has been intensively studied, but the conclusions are quite inconsistent [33–39]. In the present study, we found that 25(OH)D levels were lower in DR patients, which was consistent with some previous studies [33, 34]. More meticulously designed studies are warranted to elucidate the relationship between vitamin D and DR.

There are some limitations to our study. Firstly, this was a single-centre study with a relatively small sample size, which might cause a bias in patient selection. Secondly, since some anti-hyperglycaemia medicines are found to possess anti-oxidative or anti-inflammatory effects, it is hard to exclude the effects of these medicines on circulating levels of Gal-3 and Fet-A. Finally, the $HbA_{1c}$ levels of patients enrolled in this study were relatively high, and due to the limited and confusing previous data, it is hard to conclude whether $HbA_{1c}$ levels may interrupt Gal-3 and Fet-A content and data interpretation. Multi-centre studies with large sample size and well-designed data stratification are warranted.

## Conclusions

Increased circulating Gal-3 and Fet-A levels are correlated with DR, which might serve as circulating biomarkers for non-invasive early diagnosis of DR. Further studies with a larger sample size may provide more information about their clinical utility in the future.

### *Data availability statement*

The datasets analysed during the present study are available from the corresponding author on reasonable request.

### *Ethics statement*

This study was proved by Ethics Committee of the Third Hospital of Hebei Medical University (Ethics certificate No. W2021-084-1) and was conducted in accordance with the principles in the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

## Author contributions

M.L., X.L.J., and Y.L. developed the manuscript concept and composed the initial draft. M.M.T. collected data. Y.L.W. analysed data. H.J.M. and Y.R.Z. contributed valuable comments on the first draft. M.L., M.M.T., Y.L.W., H.J.M., Y.R.Z., X.L.J., and Y.L. critically revised the manuscript for intellectual content. All authors read and approved the final manuscript version to be published.

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## Conflict of interest

The authors declare that they have no conflict of interest.

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