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Apolipoprotein and menopausal status are significant influencing factors for diabetic retinopathy in type II diabetes mellitus women

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Diabetic retinopathy (DR) is a common complication of type II diabetes mellitus (T2DM) and a leading cause of blindness in the working population. Apolipoprotein levels have been reported to be associated with the risk of DR. This study aimed to develop a predictive model for DR based on apolipoproteins (apoA and apoB) and menopausal status in Chinese Han women with T2DM and to evaluate the model's effectiveness. Data from 2339 T2DM women were collected between January 2018 and June 2022. Multilevel regression was used to explore the independent effect of apolipoproteins and interaction between apolipoproteins and menopausal status on DR and proliferative diabetic retinopathy (PDR). Receiver operating characteristic (ROC) analysis was performed to compare the fitting degree and predictive efficiency of different models. Results showed that both apoA and apoB were independent influencing factors for DR and PDR and interacted significantly with menopausal status. The interaction between apoA and menopausal status had a protective effect on DR [OR (95% CI) = 0.925 (0.858–0.996), *P* = 0.040] and PDR [OR (95% CI) = 0.937 (0.895–0.981), *P* = 0.006]. In contrast, the interaction between apoB and menopausal status was a risk factor for DR [OR (95% CI) = 1.684 (1.141–2.379), *P* = 0.008)] and PDR [OR (95% CI) = 3.377 (1.148–9.937), *P* = 0.027]. ROC analysis demonstrated that the interaction model outperformed models without interaction terms (*P* < 0.01). The area under the curve for the interaction model was 0.879 (95% CI 0.864–0.893) for DR and 0.930 (95% CI 0.915–0.945) for PDR. These findings suggest that the interaction model is highly efficient in predicting DR, particularly PDR, in Chinese Han women with T2DM.

Keywords Diabetic retinopathy, Apolipoprotein, Menopausal status, Type II diabetes mellitus, Predictive modeling, Interaction effects

Abbreviations

apoA	Apolipoprotein A
apoB	Apolipoprotein B
AUC	Area under the receiver operating characteristic curve
CI	Confidence interval
DBP	Diastolic blood pressure
DM	Diabetes mellitus
DR	Diabetic retinopathy
HDL	High-density lipoprotein
HbA1c	Hemoglobin A1c
ICDR	International clinical diabetic retinopathy
IQR	Inter-quartile range
LDL	Low-density lipoprotein
Lpa	Lipoprotein (a)
NDR	Non-diabetic retinopathy
OCTA	Optical coherence tomography angiography

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OR	Odds ratio
RGCs	Retinal ganglion cells
ROC	Receiver operator characteristic
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SD	Standard deviation
T2DM	Type II diabetes mellitus
TC	Total cholesterol
TG	Triglyceride

Background

Diabetic retinopathy (DR) is one of the most prevalent complications of type II diabetes mellitus (T2DM) and represents the leading cause of blindness among the adult working population[1,2]. Beyond its detrimental effects on vision, DR significantly increases the risk of cardiovascular diseases. By 2025, the global prevalence of DR is projected to rise to 5.4%[3]. According to the International Diabetes Federation (2021), there are 300 million women worldwide with diabetes mellitus (DM), representing a prevalence rate of 10.2%[4]. In China, the prevalence of DR in women with DM is notably higher at 29.8%[5]. DR has thus become a critical public health challenge globally[6]. Its onset is often insidious, and delayed diagnosis can lead to vision-threatening complications. Furthermore, the endocrinology departments, where most T2DM patients first seek care, are often insufficiently equipped to diagnose DR early. This underscores the urgent need for sensitive biomarkers to facilitate timely detection of DR.

T2DM is a systemic metabolic disorder frequently accompanied by dyslipidemia[7,8]. Dyslipidemia accelerates pathological processes such as non-enzymatic glycosylation and polyol pathway activation, which contribute to microvascular basement membrane thickening, endothelial dysfunction, microcirculatory disturbances, retinal barrier damage, and other DR-related pathological changes[9,10]. Compared to DM patients without dyslipidemia, those with dyslipidemia exhibit a higher prevalence of DR[11–13]. Lipoprotein, apolipoproteins, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and total cholesterol (TC) are strongly associated with the incidence and severity of DR[12,14]. Apolipoproteins, which mediate cholesterol transport from blood to the liver, play a pivotal role in lipid metabolism and are linked to an elevated risk of coronary heart disease, stroke, and DR[12,15,16].

Additionally, menopause has been shown to influence apolipoprotein levels and DR prevalence[17,18]. The rapid decline in estrogen levels among postmenopausal women contributes to dyslipidemia and diminishes the protective effects on retinal ganglion cells (RGCs)[18,19]. Despite these findings, no prior studies have explored whether the interaction between apolipoproteins and menopausal status affects DR.

This cross-sectional study aims to investigate the relationship between apolipoproteins and menopausal status in relation to DR in women with T2DM. Furthermore, an interaction model was developed to evaluate the combined effects of apolipoproteins and menopausal status on DR. Receiver operator characteristic (ROC) analysis was conducted to assess the model's fitting degree and prediction efficiency.

Materials and methods

Participants

This cross-sectional study included female patients with T2DM who were hospitalized in the Endocrinology Departments of Henan Provincial People's Hospital, Guangshan County People's Hospital, and Xincai County People's Hospital between January 2018 and December 2021.

Inclusion criteria Female patients aged ≥ 18 years with T2DM diagnosed according to the 2018 standards of the American Diabetes Association, which include fasting plasma glucose ≥ 7.0 mmol/L, oral glucose tolerance test two-hour plasma glucose ≥ 11.1 mmol/L, HbA1c ≥ 6.5%, or self-reported T2DM. Participants were required to provide informed consent and agree to participate in the study.

Exclusion criteria Patients were excluded if they had other retinal diseases that could be confused with DR, such as hypertensive retinal disease, retinal vasculitis, or retinal vein occlusion. Exclusion also applied to patients lacking key data, including age, menopausal status, or blood lipid measurements, as well as those with unclear fundus photos due to small pupils, cataracts, or vitreous opacity.

This study adhered to the principles outlined in the Declaration of Helsinki and was approved by the Ethics Committee of Henan Provincial People's Hospital [ethical approval code: HNEECKY-2022(22)]. All participants were fully informed about the purpose and details of the study and provided written informed consent.

The sample size was calculated using PASS 11.0 software, based on formulas for cross-sectional studies (formula a) and diagnostic tests (formula b)[20]. The significance level (α) was set at 0.05, statistical power at 0.90, and allowable error (δ) at 0.08. In formula (a), k represented the ratio of patients without DR to those with DR, with k = 1 in this study. In formula (b), p represented the sensitivity or specificity of the diagnostic method. Pre-survey results indicated that the mean apoA levels in T2DM patients with and without DR were 1.16 ± 0.37 g/L and 1.07 ± 0.43 g/L, respectively, and the mean apoB levels were 0.96 ± 0.38 g/L and 0.89 ± 0.41 g/L, respectively. The sensitivity and specificity of the diagnostic method were 80.3% and 78.6%, respectively. Based on these parameters, the required sample size was 418 for both the DR and non-DR groups. To account for potential invalid samples, a 20% margin was added, resulting in a minimum required sample size of 502 for each group.

$$n = \frac{(Z_\alpha + Z_\beta)^2(1 + 1/k)\sigma^2}{\delta^2} \quad (1)$$

$$n = \left(\frac{z_\alpha}{\delta}\right)^2 (1-p)p \quad (2)$$

Data collection

At the beginning of the study, a self-designed questionnaire was used to collect information from participants, including demographic characteristics, menopausal status, age at the onset of menopause, medical history, and family history. Fundus examinations were conducted by an experienced ophthalmologist using a Zeiss non-mydriatic fundus camera (VISUCAM 224, Germany). Images were captured from five fields of each eye: macula-centered, temporal, nasal, upper quadrant, and lower quadrant of the retina. Additionally, optical coherence tomography angiography (OCTA) was performed to evaluate retinal microvascular changes in the T2DM patients.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after 10 min of seated rest. Measurements were taken three times by the same staff using a calibrated M7 digital sphygmomanometer (Omron M7, Hoofddorp, The Netherlands), and the average of the three readings was recorded.

Blood samples were collected from fasting veins in the morning, and serum samples were separated to measure levels of lipoprotein (a) (Lpa), apolipoprotein A (apoA), apolipoprotein B (apoB), HDL, LDL, triglyceride (TG), TC, and hemoglobin A1c (HbA1c). Clinical data and biochemical indicator values were retrieved from the hospital information system and independently checked by two authors to ensure accuracy.

Definitions and diagnostic criteria

The diagnosis of DR was determined by an ophthalmologist with more than five years of clinical experience based on the International Clinical Diabetic Retinopathy (ICDR) criteria[21]. DR was classified into three categories: (1) Absence of DR (NDR): no visible signs of retinopathy or abnormalities; (2) Non-proliferative diabetic retinopathy (NPDR): the early stage of DR characterized by leaking blood vessels or fluid within the retina without the formation of new blood vessels; and (3) Proliferative diabetic retinopathy (PDR): an advanced stage of DR involving the growth of abnormal and fragile new blood vessels triggered by retinal signals (neovascularization). "Any DR" includes both NPDR and PDR. In cases where grading results from two clinicians were inconsistent, a panel of four retina specialists adjudicated to establish the reference standard. Patients with DR in both eyes were treated as a single case, and the severity of DR was graded according to the more severely affected eye. Menopause was defined as meeting any of the following criteria: (a) amenorrhea for ≥ 12 months; (b) age ≥ 60 years; or (c) a history of bilateral ovariectomy in T2DM patients[22].

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 25.0 (IBM, Chicago, IL) and R software (version 4.2.0). Quantitative data were described as mean ± standard deviation (SD) for normal distribution data and median (inter-quartile range, IQR) for skewed data. Quantitative data were compared by *t*-test (normal distribution data) or nonparametric test (skewed data). Qualitative data were described by frequency and were compared by chi-square test. Multilevel regression analysis was employed to evaluate the relationships between apolipoproteins, menopausal status, and DR. Interaction models were constructed to examine the combined effects of apolipoproteins and menopausal status on DR. Forest plots of the multilevel regression results were generated using R software (version 4.2.0). The importance ranking of variables and Shapley Additive Explanations (SHAP) dependency plots were generated based on a random forest model, using the "randomForest," "shapviz," and "randomForestExplainer" packages in R software (version 4.2.0). ROC analysis was performed to evaluate the model's fitting degree and predictive efficiency for DR and PDR. ROC curves were generated using IBM SPSS Statistics 25.0 (IBM, Chicago, IL). The DeLong test was applied to compare the area under the curve (AUC) between ROC curves. All statistical tests were two-sided, with a significance level of $\alpha = 0.05$.

Results

Participant characteristics

According to the inclusion criteria, a total of 2463 female T2DM patients were screened. Their personal information and clinical data were collected, and 124 patients were excluded because of incomplete information or unclear fundus photos. Therefore, 2339 patients aged 18–92 (58.17 ± 13.87) were analyzed. Among them, 1621 patients (69.3%) were postmenopausal, and 718 (30.7%) were premenopausal. The duration of T2DM was 9.69 ± 7.48 years. There were 925 (including 635 NPDR and 290 PDR) DR patients and 1414 (60.5%) non-diabetic retinopathy (NDR) patients. The general characteristics and biochemical indicators of the two groups are shown in Table 1.

Association between apolipoprotein and DR in premenopausal and postmenopausal women with T2DM

Our preliminary analysis indicated that the relationships between apoA, apoB, and the prevalence of DR were non-linear. Compared to analyzing these variables as continuous numerical data, converting apoA and apoB into categorical variables based on quartiles allowed us to more accurately capture their associations with DR. Consequently, all patients were divided into four groups according to the quartile of apoA ($apoA_{P1}$: apoA ≤ 1.07 g/L, $apoA_{P2}$: 1.08 g/L ≤ apoA ≤ 1.19 g/L, $apoA_{P3}$: 1.20 g/L ≤ apoA ≤ 1.36 g/L, $apoA_{P4}$: apoA ≥ 1.37 g/L) and apoB ($apoB_{P1}$: apoB ≤ 0.78 g/L, $apoB_{P2}$: 0.79 g/L ≤ apoB ≤ 0.98 g/L, $apoB_{P3}$: 0.99 g/L ≤ apoB ≤ 1.22 g/L, $apoB_{P4}$: apoB ≥ 1.23 g/L). The prevalence of DR and PDR was analyzed across these groups (Fig. 1A, B).

Variable	NDR (n = 1414)	DR (n = 925)
Postmenopausal, n (%) [a]	918 (64.9)	703 (76.0)***
Hypertension, n (%)[a]	606 (42.9)	515 (55.7)***
Age, years old, mean ± SD[b]	57.87 ± 13.44	60.24 ± 14.08***
Duration of T2DM, years, median (IQR)[c]	7.00 (2.00–10.25)	13.00 (7.00–20.00)***
apoA, g/L, median (IQR)[c]	1.19 (1.03–1.35)	1.19 (1.08–1.36)**
apoB, g/L, median (IQR)[c]	0.97 (0.78–1.22)	1.02 (0.81–1.21)**
Lpa, mg/L, median (IQR)[c]	115.00 (60.00–244.00)	122.00 (78.00–223.00)*
HDL, mmol/L, median (IQR)[c]	1.04 (0.88–1.19)	1.06 (0.90–1.33)***
LDL, mmol/L, median (IQR)[c]	3.43 (2.72–3.98)	3.61 (2.83–4.13)***
TG, mmol/L, median (IQR)[c]	1.81 (1.31–2.65)	1.77 (1.17–2.42)*
TC, mmol/L, median (IQR)[c]	5.20(4.30–6.00)	5.60 (4.50–6.30)***
HbA1c, %, median (IQR)[c]	9.40 (7.65–11.20)	9.90 (8.10–11.50)***

Table 1. General characteristics and biochemical indicators of the DR group and the NDR group (N = 2339) *NDR* non-diabetic retinopathy, *DR* diabetic retinopathy, *SD* standard deviation, *IQR* inter-quartile range, *Lpa* lipoprotein, apoA apolipoprotein A, *apoB* apolipoprotein B, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *TG* triglyceride, *TC* total cholesterol, *HbA1c* Hemoglobin A1c, a-c: comparison between DR and NDR group using Chi-square test (a), t-test (b), and nonparametric test (c); *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

In premenopausal women, the prevalence of both DR and PDR was significantly higher in the highest apoA quartile ($apoA_{P4}$) compared to the second and third quartiles ($apoA_{P2}$ and $apoA_{P3}$) [(any DR: $apoA_{P2}$ vs. $apoA_{P4}$: 25.0% vs. 44.4%, $P = 0.001$; $apoA_{P3}$ vs. $apoA_{P4}$: 27.5% vs. 44.4%, $P = 0.002$), (PDR: $apoA_{P2}$ vs. $apoA_{P4}$: 12.5% vs. 25.9%, $P = 0.005$; $apoA_{P3}$ vs. $apoA_{P4}$: 9.2% vs. 25.9%, $P < 0.001$)]. Similarly, the prevalence was higher in the lowest apoB quartile ($apoB_{P1}$) compared to the third and fourth quartiles ($apoB_{P3}$ and $apoB_{P4}$) [(any DR: $apoB_{P1}$ vs. $apoB_{P3}$: 43.8% vs. 33.0%, $P = 0.036$; $apoB_{P1}$ vs. $apoB_{P4}$: 43.8% vs. 25.0%, $P < 0.001$), (PDR: $apoB_{P1}$ vs. $apoB_{P3}$: 22.5% vs.11.4%, $P = 0.005$; $apoB_{P1}$ vs. $apoB_{P4}$: 22.5% vs. 14.1%, $P = 0.040$)].

In postmenopausal women, different patterns were observed. The prevalence of DR and PDR was lower in the lowest ($apoA_{P1}$) and highest ($apoA_{P4}$) apoA quartiles compared to the second ($apoA_{P2}$) and third ($apoA_{P3}$) quartiles [(any DR: $apoA_{P1}$ vs. $apoA_{P2}$: 37.6% vs. 51.5%, $P < 0.001$; $apoA_{P1}$ vs. $apoA_{P3}$: 37.6% vs. 47.4%, $P = 0.006$; $apoA_{P2}$ vs. $apoA_{P4}$: 51.5% vs. 37.8%, $P < 0.001$; $apoA_{P3}$ vs. $apoA_{P4}$: 47.4% vs. 37.8%, $P = 0.005$), (PDR: $apoA_{P1}$ vs. $apoA_{P2}$: 7.4% vs. 14.6%, $P = 0.001$; $apoA_{P1}$ vs. $apoA_{P3}$: 7.4% vs. 15.1%, $P = 0.001$; $apoA_{P2}$ vs. $apoA_{P4}$: 14.6% vs. 8.7%, $P = 0.006$; $apoA_{P3}$ vs. $apoA_{P4}$: 15.1% vs. 8.7%, $P = 0.004$)]. Conversely, the prevalence was lower in the first ($apoB_{P1}$) and second ($apoB_{P2}$) apoB quartiles compared to the fourth quartile ($apoB_{P4}$) [(any DR: $apoB_{P1}$ vs. $apoB_{P4}$: 31.5% vs. 49.7%, $P < 0.001$; $apoB_{P2}$ vs. $apoB_{P4}$: 40.9% vs. 49.7%, $P = 0.015$), (PDR: $apoB_{P1}$ vs. $apoB_{P4}$: 8.8% vs. 18.5%, $P < 0.001$; $apoB_{P2}$ vs. $apoB_{P4}$: 10.0% vs. 18.5%, $P = 0.001$)].

These results suggest that the effects of apoA and apoB on DR and PDR differ between premenopausal and postmenopausal women with T2DM. Specifically, in premenopausal women, the relationship between apoA and DR/PDR prevalence exhibited a U-shaped pattern, while apoB showed an L-shaped pattern. In postmenopausal women, apoA and DR/PDR prevalence demonstrated an inverted U-shaped pattern, whereas apoB followed a J-shaped pattern.

The correlation between apoA and apoB

Spearman correlation analysis was performed to evaluate the relationship between apoA and apoB levels. The Spearman correlation coefficients (r_s) are summarized in Table 2. The analysis revealed no significant linear correlation between apoA and apoB in premenopausal women with T2DM ($r_s = -0.043$, $P = 0.254$). However, a weak but statistically significant correlation was observed in the overall study population ($r_s = 0.041$, $P = 0.045$) and in postmenopausal women with T2DM ($r_s = 0.077$, $P = 0.002$). When stratified by DR status, the correlation between apoA and apoB was stronger in patients with DR compared to those without DR (NDR), with the highest correlation observed in PDR patients. These findings suggest that the relationship between apoA and apoB is influenced by both menopausal status and the presence or severity of DR.

Apolipoprotein and menopausal status as significant factors for DR in women with T2DM

To investigate the influence of apolipoproteins and menopausal status on DR, we constructed five multilevel regression models using DR and PDR as dependent variables. The independent variables and covariates included in these models are detailed in Table 3. In Models 2, 4, and 5, menopausal status was treated as a within-subject variable. Forest plots illustrating the multilevel regression results are presented in Fig. 2A, B. When apoA and apoB were analyzed independently (Models 1 and 3), their effects on DR were relatively small. However, after incorporating menopausal status and its interaction with these apolipoproteins, their influence on DR increased significantly (Models 2 and 4). This indicates that apoA and apoB not only directly affect DR prevalence but also indirectly influence it through their interaction with menopausal status. In the comprehensive Model 5, which included apoA, apoB, menopausal status, and interaction terms, the results showed that apoA levels of ≥ 1.20 g/L were associated with an increased risk of DR (1.20 g/L ≤ apoA ≤ 1.36 g/L: OR (95% CI) = 1.900 (1.047–3.448),

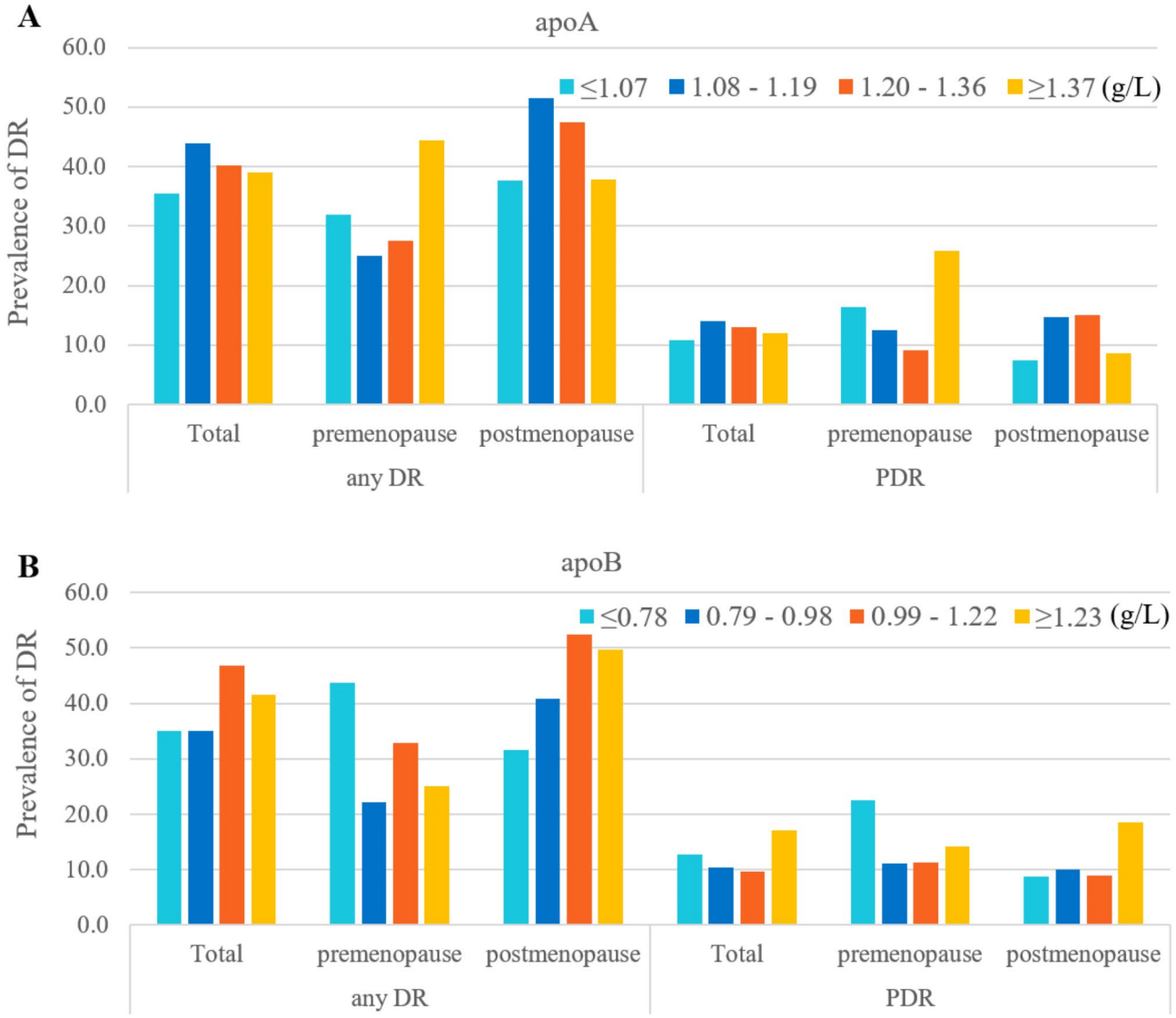

Fig. 1. The relationship between apolipoprotein and prevalence of DR. **A** The relationship between apoA and prevalence of DR (left: any DR; right: PDR); **B** The relationship between apoB and prevalence of DR (left: any DR; right: PDR)

$P = 0.035$; apoA ≥ 1.37 g/L: OR (95% CI) = 3.881 (1.988–7.579), $P < 0.001$) and PDR (1.20 g/L ≤ apoA ≤ 1.36 g/L: OR (95% CI) = 2.394 (1.248–4.593), $P = 0.009$; apoA ≥ 1.37 g/L: OR (95% CI) = 7.798 (3.061–19.867), $P < 0.001$). The interaction between apoA and menopausal status demonstrated a weak protective effect on DR [OR (95% CI) = 0.925 (0.858–0.996), $P = 0.040$] and PDR [OR (95% CI) = 0.937(0.895–0.981), $P = 0.006$]. Similarly, higher apoB levels (≥ 1.23 g/L) were associated with an increased prevalence of DR [OR (95% CI) = 1.594 (1.135–2.240), $P = 0.007$] and PDR [OR (95% CI) = 1.747 (1.051–2.905), $P = 0.031$]. The interaction between apoB and menopausal status had an adverse effect on DR [OR (95% CI) = 1.648 (1.141–2.379), $P = 0.008$] and PDR [OR (95% CI) = 3.377 (1.148–9.937), $P = 0.027$]. Postmenopausal women were at a higher risk of DR [OR (95% CI) = 1.843 (1.517–2.239), $P < 0.001$] and PDR [OR (95% CI) = 1.078 (1.008–1.154), $P = 0.027$] compared to premenopausal women. Additionally, the interaction between apoA and age showed adverse effects on DR [OR (95% CI) = 1.034 (1.028–1.041), $P < 0.001$] and PDR [OR (95% CI) = 1.108 (1.037–1.183), $P = 0.003$].

A random forest model was also utilized to determine the importance of independent variables for DR and PDR. The ranking of feature importance based on accuracy is depicted in Fig. 3A (DR) and Fig. 3B (PDR). These results highlighted apoA, apoB, and menopausal status as critical factors, with apoA having a greater impact than apoB. Based on the random forest model, SHAP values were computed to visualize the effects of these variables, and the dependence plots are shown in Fig. 3C–F. The results indicated a non-linear relationship between apoA and DR, with interactions between lower and higher apoA levels and menopausal status exhibiting contrasting effects. Specifically, low and high apoA levels combined with non-menopausal status had a positive effect on DR, while the same levels combined with menopausal status had a negative effect (Fig. 3C, D). Conversely, apoB displayed an approximately linear relationship with DR, and higher apoB levels combined with menopausal

		r_s	*P*
Total	All	0.041	0.045
	NDR	− 0.017	0.512
	Any DR	0.135	< 0.001
	PDR	0.151	0.010
Premenopausal	All	− 0.043	0.254
	NDR	− 0.139	0.002
	Any DR	0.152	0.023
	PDR	− 0.036	0.713
Postmenopausal	All	0.077	0.002
	NDR	0.059	0.016
	Any DR	0.114	0.002
	PDR	0.267	< 0.001

Table 2. The Spearman correlation coefficient between apoA and apoB *NDR* non-diabetic retinopathy, *DR* diabetic retinopathy, *PDR* proliferative diabetic retinopathy, r_s Spearman correlation coefficient

	Independent variables
Model 1	apoA + covariates
Model 2	apoA + menopausal status + apoA*menopausal status + apoA*age + covariates
Model 3	apoB + covariates
Model 4	apoB + menopausal status + apoB*menopausal status + apoB*age + covariates
Model 5	apoA + apoB + menopausal status + apoA*menopausal status + apoB*menopausal status + apoA*age + apoB*age + covariates

Table 3. The independent variables of five regression models In five models, covariates include age, hypertension, duration of T2DM, Lpa, HDL, LDL, TG, TC and HbA1c

status amplified DR risk (Fig. 3E, F). These findings, consistent with the multilevel regression analysis, suggest that apoA, apoB, menopausal status, and their interactions are significant factors influencing DR and PDR in women with T2DM.

The fitting degree and prediction efficiency of the five models in women with T2DM

To evaluate the fitting degree and prediction efficiency of the five models, we used DR and PDR as state variables and the prediction probabilities of the models as test variables to construct ROC curves (Fig. 4A, B). The ROC analysis revealed that models incorporating menopausal status and interaction effects had a significantly better fit compared to models without these variables. Specifically, the comparison between models 1 and 2 showed an improvement in the AUC (0.671 vs. 0.727, $Z = 3.600$, $P < 0.001$). Similarly, the AUC for model 4, which included interaction effects, was significantly higher than that of model 3 (0.703 vs. 0.661, $Z = 2.700$, $P = 0.007$). Model 5, which included both apoA and apoB along with menopausal status and their interactions, demonstrated the best fitting degree among all models. The AUC of model 5 was significantly higher compared to model 2 (0.879 vs. 0.727, $Z = 11.658$, $P < 0.001$) and model 4 (0.879 vs. 0.703, $Z = 13.499$, $P < 0.001$), for both any DR and PDR. Additionally, model 5 showed superior predictive efficiency for PDR compared to any DR, with an AUC of 0.930 for PDR versus 0.879 for any DR ($Z = 4.798$, $P < 0.001$). The results of the five model in predicting DR and PDR are shown in Table 4. These findings suggest that incorporating the interaction between apolipoproteins and menopausal status significantly enhances the model's ability to explain variations in DR and PDR, providing a more robust predictive model.

Discussion

Apolipoproteins, primarily synthesized in the liver, combine with lipids to form lipoproteins, which are crucial for lipid metabolism and transportation. These processes play a significant role in regulating blood lipid levels[23,24]. Apolipoproteins have been shown to be closely associated with the occurrence and progression of DR[12]. This study provides evidence that in female patients with T2DM, the effects of apoA and apoB on DR and PDR differ between premenopausal and postmenopausal women. Specifically, the interaction between apolipoproteins and menopausal status significantly influences the prevalence of both any DR and PDR. Our findings demonstrate that incorporating the interaction between apoA, apoB, and menopausal status into regression models significantly improves the explanation of variations in DR and PDR. The models that included interaction terms outperformed those without interactions in terms of fitting degree and predictive efficiency. Notably, the prediction efficiency for PDR in the interaction model was superior to that for any DR. These results highlight the importance of considering both apolipoproteins and menopausal status, along with their interactions, when studying the risk factors for DR and PDR in women with T2DM. This approach provides

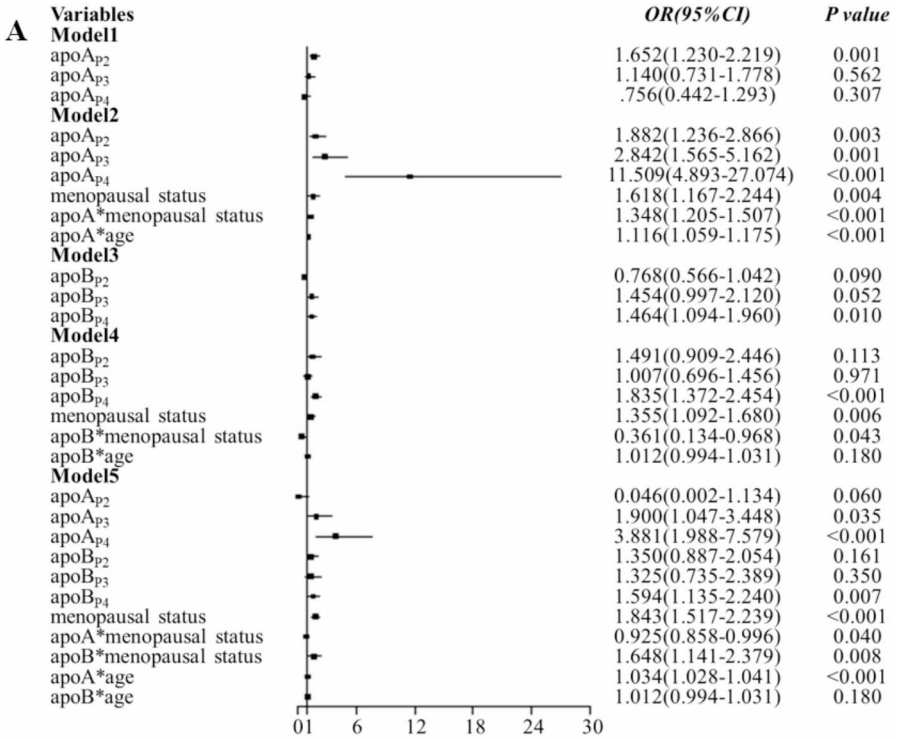

Fig. 2. Multilevel regression forest plot of DR and influencing factors. **A** Multilevel regression forest plot of the five models for any DR; **B** Multilevel regression forest plot of the five models for PDR. Results were adjusted by age, hypertension, duration of T2DM, Lpa, HDL, LDL, TG, TC, HbA1c.

a more nuanced understanding of the mechanisms underlying these conditions and could contribute to more targeted screening and intervention strategies.

This study investigated the influence of apolipoproteins and menopausal status on the prevalence of DR and PDR in female patients with T2DM using statistical descriptions and multilevel regression analyses, incorporating interaction effects. The findings revealed that the relationship between apolipoproteins and DR

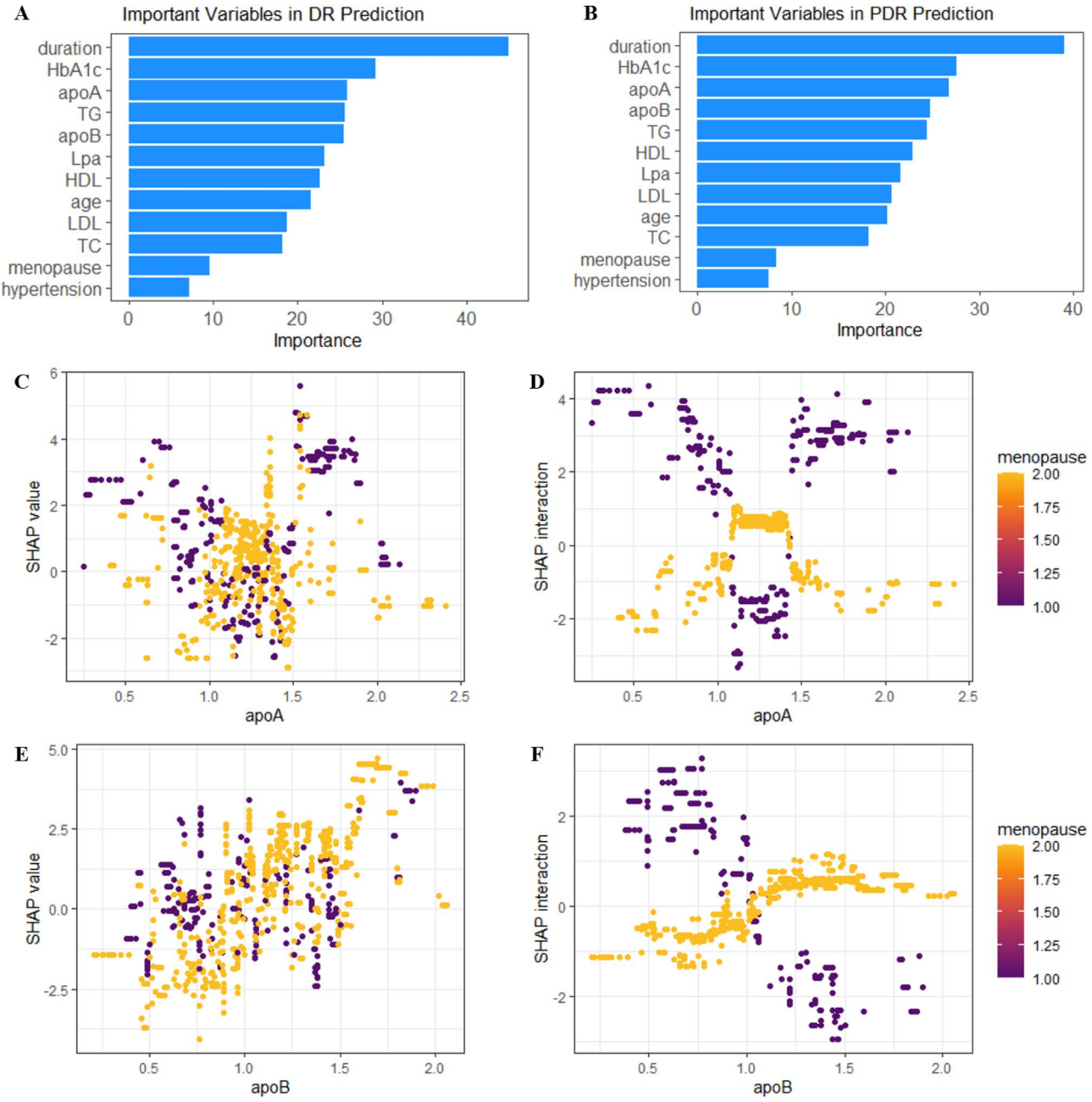

Fig. 3. The importance and SHAP value of apoA and apoB based on random forest model. **A** The importance of apoA, apoB, and other independent variables on the dependent variable (DR) in the random forest model; **B** The importance of apoA, apoB, and other independent variables on the dependent variable (PDR) in the random forest model; **C** SHAP dependency plot of apoA on DR based on random forest model, purple represents premenopausal individuals, while yellow represents postmenopausal individuals; **D** SHAP dependent plot of the interaction effect between apoA and menopause on DR; **E** SHAP dependency plot of apoB on DR based on random forest model, purple represents premenopausal individuals, while yellow represents postmenopausal individuals; **F** SHAP dependent plot of the interaction effect between apoB and menopause on DR

varied significantly between premenopausal and postmenopausal patients and that apolipoproteins exerted distinct effects on DR at different levels. In premenopausal women, those with apoA levels ≥ 1.37 g/L had a higher risk of DR compared to those with apoA levels < 1.37 g/L, while those with apoB levels ≤ 0.78 g/L exhibited a higher risk of DR than those with apoB levels > 0.78 g/L. In contrast, in postmenopausal women, those with apoA levels > 1.07 g/L but < 1.37 g/L faced a higher risk of DR compared to those with apoA levels ≤ 1.07 g/L or ≥ 1.37 g/L. Similarly, postmenopausal women with apoB levels ≤ 0.98 g/L had a lower risk of DR than those with levels > 0.98 g/L. These findings underscore the importance of routine monitoring of apolipoprotein levels

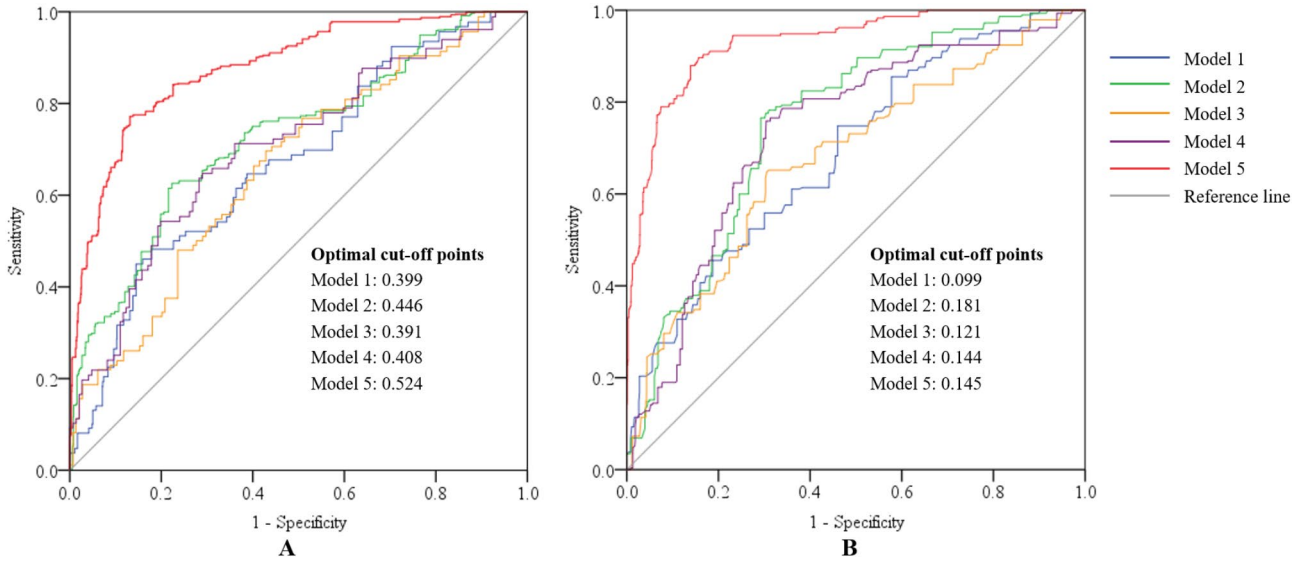

Fig. 4. ROC curves for five models predicting DR in women with T2DM. **A** ROC curves for five model predicting any DR; **B** ROC curves for five model predicting PDR

	Sensitivity (%)	Specificity (%)	Cut-off	Accuracy (%)	AUC
Any DR					
Model1	60.4	63.6	0.399	62.4	0.671 (0.649–0.694)
Model2	62.6	77.7	0.446	71.7	0.727 (0.705–0.748)
Model3	62.3	61.2	0.391	61.7	0.661 (0.638–0.683)
Model4	64.8	71.0	0.408	68.5	0.703 (0.681–0.725)
Model5	77.2	86.8	0.524	83.0	0.879 (0.864–0.893)
PDR					
Model1	74.8	54.0	0.099	56.6	0.690 (0.658–0.721)
Model2	76.6	70.8	0.181	71.5	0.756 (0.730–0.783)
Model3	66.6	58.9	0.121	59.9	0.680 (0.646–0.714)
Model4	75.9	69.6	0.144	70.4	0.737 (0.707–0.766)
Model5	87.9	86.1	0.145	86.3	0.930 (0.915–0.945)

Table 4. The results of the five model in predicting DR and PDR *DR* diabetic retinopathy, *PDR* proliferative diabetic retinopathy, *AUC* area under the receiver operating characteristic curve

in T2DM women. For premenopausal women, fundus examinations should be performed when apoA levels are ≥ 1.37 g/L and apoB levels are ≤ 0.78 g/L. For postmenopausal women, fundus function should be evaluated when apoA levels exceed 1.07 g/L or apoB levels exceed 0.98 g/L to detect and address potential DR early.

Multilevel regression analyses showed that, without considering menopausal status, apoA and apoB had relatively small effects on DR prevalence. However, when menopausal status and its interaction with apolipoproteins were included, the impact of both apoA and apoB on DR increased significantly. The comprehensive Model 5 demonstrated that apoA, apoB, menopausal status, and their interactions all significantly influenced DR prevalence. While menopause and the interaction between apoB and menopausal status were identified as risk factors for DR and PDR, the interaction between apoA and menopausal status acted as a protective factor for both conditions. The predictive performance of the five models was also evaluated, with Model 5 achieving the highest AUC values of 0.879 for any DR and 0.930 for PDR. The accuracy of Model 5 was 83.0% for DR and 86.3% for PDR. These findings suggest that the interaction model is a valuable tool for predicting DR in women, particularly for identifying those at risk for PDR.

DR is a common microvascular complication of T2DM. Patients with T2DM and DR face an elevated risk of cardiovascular complications, including stroke and coronary heart disease[25–28]. Given these serious health implications, identifying reliable biomarkers for DR holds significant clinical importance. This study demonstrated that dyslipidemia, specifically abnormal levels of apoA and apoB, is statistically associated with DR prevalence. Moreover, the relationship between apolipoproteins and DR varied based on menopausal status, suggesting a complex interplay between lipid profiles and hormonal factors in DR pathogenesis.

Comparisons with previous studies underscore the novelty of these findings. A cross-sectional study by Ankit et al. conducted in India reported that HDL and apoAI levels were inversely related to DR severity, while

apoB levels were positively correlated with DR severity in a cohort of 117 DR patients[29]. Consistent with these observations, our study confirmed associations between apolipoproteins and DR but highlighted differences between premenopausal and postmenopausal women, revealing unique patterns that were not explored in earlier research. Similarly, a retrospective cohort study of 1023 diabetes patients found that baseline serum apoAI levels ≥ 7.4 μmol/L were associated with a reduced risk of DR, while elevated levels of apoCIII (≥ 6.3 μmol/L), apoE (≥ 1.1 μmol/L), and ratios such as apoCIII/apoAI (≥ 0.9) and apoE/apoAI (≥ 0.2) were linked to an increased risk of DR[30]. Additionally, a case-control study by Moosaie et al. demonstrated a negative correlation between serum apoA and DR risk in T2DM patients[12]. While these studies support the role of apolipoproteins in DR, our findings provide deeper insights by stratifying results based on menopausal status, emphasizing the influence of hormonal changes on apolipoprotein effects. These distinctions advance the understanding of apolipoprotein-related dyslipidemia as a potential biomarker for DR, particularly in the context of menopausal transitions in women with T2DM.

Previous studies have suggested that elevated levels of apoA and its subtypes may act as protective factors against DR, while apoB has been positively correlated with DR severity. However, our findings diverge from these patterns. In premenopausal women with T2DM, apoA levels ≥ 1.37 g/L were identified as a risk factor for DR, while lower apoB levels emerged as a risk factor. In postmenopausal women, lower apoB levels became a protective factor. Several factors may explain these differences. First, the outcome in our study focused on the prevalence of DR, whereas studies by Ankit et al. and Moosaie et al. examined DR severity. This distinction in study endpoints likely contributes to the observed discrepancies. Second, the influence of estrogen on the pathophysiology of DR provides a compelling explanation. In hyperglycemic environments, estrogen, specifically 17β-estradiol, stabilizes the mitochondrial membrane potential in RGCs, reduces intracellular reactive oxygen species (ROS) levels, upregulates anti-apoptotic protein Bcl-2, and inhibits pro-apoptotic protein Bax. These processes collectively protect retinal ganglia and reduce cell apoptosis. Before menopause, the presence of estrogen may antagonize the adverse effects of apolipoproteins on DR. This protective effect diminishes after menopause due to the rapid decline in estrogen levels, potentially explaining the increased prevalence of DR in postmenopausal women[18]. In contrast, prior studies did not account for the modulatory role of estrogen, highlighting a critical gap that our findings address. These results underscore the importance of considering hormonal influences when evaluating the relationship between apolipoproteins and DR, particularly in the context of menopausal transitions. Such insights can inform more targeted approaches to the prevention and treatment of DR in women with T2DM.

Most previous studies examining the relationship between cholesterol or apolipoprotein levels and DR prevalence did not consider the potential impact of menopausal status. In contrast, our study separately analyzed the associations between apolipoproteins and DR in premenopausal and postmenopausal women. Additionally, we assessed the interaction between apolipoproteins and menopausal status using multilevel regression models. The results of our ROC analysis demonstrated that regression models incorporating interaction terms outperformed those without interactions, underscoring the importance of accounting for the interplay between apolipoproteins and menopausal status. Furthermore, the combined action model of apoA and apoB exhibited superior predictive performance compared to models evaluating apoA or apoB independently. This finding suggests that apolipoproteins influence DR not only independently but also indirectly through their interactions with sex hormones.

Moreover, DR patients often first visit endocrinology departments, where the use of fundus cameras and OCTA may not be routine. Consequently, DR is frequently underdiagnosed in its early stages. The measurement of apoA and apoB, however, is a standard part of biochemical testing in endocrinology clinics. This routine assessment does not increase patients' medical expenses and could serve as an auxiliary diagnostic tool for identifying patients at higher risk for DR. Our findings highlight the practical significance of integrating apolipoprotein levels into DR screening protocols, especially in endocrinology settings. This approach could improve the timely diagnosis and management of DR, particularly in women with T2DM.

However, this study has several limitations that should be acknowledged. First, while apolipoproteins were the primary focus of this analysis, other factors influencing the prevalence of DR, such as comorbid conditions, glycemic and lipid control, lipid management practices, self-compensation levels, and lifestyle variables, were not included. The omission of these variables may have introduced bias into the results. Second, menopausal status in this study was defined based on age, amenorrheic duration, and a history of bilateral ovariectomy. This definition did not account for hormonal markers such as follicle-stimulating hormone and estradiol levels, which could provide a more precise classification of menopausal status. Lastly, the cross-sectional design of this study limits its ability to establish causal relationships between apolipoprotein levels and DR prevalence. It remains unclear whether elevated apolipoprotein levels increase the risk of DR or whether DR itself leads to changes in apolipoprotein levels. To address these limitations, future research should include prospective studies with larger sample populations. Such studies should incorporate additional confounding variables, utilize more precise definitions of menopausal status based on hormonal levels, and explore the causal pathways between apolipoprotein levels and DR development.

Conclusion

Apolipoprotein levels and menopausal status are significant factors influencing the prevalence of DR in women with T2DM. Apolipoproteins independently affect DR, while their interaction with menopausal status also plays a critical role. Regression models that include these interaction effects demonstrate superior fit and predictive efficiency compared to models without interactions, particularly in predicting PDR. These findings underscore the importance of incorporating apolipoproteins and menopausal status into risk assessment models for DR. They provide valuable insights for early diagnosis and timely intervention, potentially improving outcomes for women with T2DM.

Data availability

All relevant data are included in the papers. Contact to corresponding author for additional information regarding data access.

Received: 4 March 2024; Accepted: 5 March 2025
Published online: 14 March 2025

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Acknowledgements

We thank all the members of our study team for their whole-hearted cooperation and the study participants for their contribution to the research.

Author contributions

ZL and XP contributed to the conception of the study. XP and XY contributed to the data interpretation, data analysis, and manuscript writing. DQ, YY and SF contributed to the data collection. All authors read and ap-

proved the final manuscript.

Funding
This work was supported by the Ministry of Science and Technology of the Peoples Republic of China [grant number 2018YFC0114500]; Henan Provincial Medical Science and Technology Research Joint Co-construction Project [grant number LHGJ20220085]; the Basic Science Project of Henan Eye Institute/Henan Eye Hospital [grant number 22JCQN004 and 21JCZD001]; and National Natural Science Foundation of China [grant number 81470603].

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
This study followed the Declaration of Helsinki and was approved by the Ethics Committee of Henan Provincial People's Hospital [ethical approval code: HNEECKY-2022(22)]. All patients were informed of the purpose and content of this study, and each one provided informed consent before being included in the study.

Consent for publication
All authors have consent for publication.

Additional information
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