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Low serum dehydroepiandrosterone levels are associated with diabetic retinopathy in patients with type 2 diabetes mellitus

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ABSTRACT

Aims: This cross-sectional study assessed the association of serum dehydroepiandrosterone levels with the risk of diabetic retinopathy in patients with type 2 diabetes mellitus in China.

Materials and Methods: Patients with type 2 diabetes mellitus were included in a multivariate logistic regression analysis to assess the association of dehydroepiandrosterone with diabetic retinopathy after adjusting for confounding factors. A restricted cubic spline was also used to model the association of serum dehydroepiandrosterone level with the risk of diabetic retinopathy and to describe the overall dose–response correlation. Additionally, an interaction test was conducted in the multivariate logistic regression analysis to compare the effects of dehydroepiandrosterone on diabetic retinopathy among age, sex, obesity status, hypertension, dyslipidemia, and glycosylated hemoglobin level subgroups.

Results: In total, 1,519 patients were included in the final analysis. Low serum dehydroepiandrosterone was significantly associated with diabetic retinopathy in patients with type 2 diabetes mellitus after adjustment for confounding factors (odds ratio [quartile 4 vs quartile 1]: 0.51; 95% confidence interval: 0.32–0.81; $P = 0.012$ for the trend). Additionally, the restricted cubic spline indicated that the odds of diabetic retinopathy decreased linearly as the dehydroepiandrosterone concentration increased (P-overall = 0.044; P-nonlinear = 0.364). Finally, the subgroup analyses showed that the dehydroepiandrosterone level stably affected diabetic retinopathy (all P for interaction >0.05).

Conclusions: Low serum dehydroepiandrosterone levels were significantly associated with diabetic retinopathy in patients with type 2 diabetes mellitus, suggesting that dehydroepiandrosterone contributes to the pathogenesis of diabetic retinopathy.

INTRODUCTION

Diabetic retinopathy (DR), a common microvascular complication of diabetes mellitus (DM), remains the leading cause of vision impairment and blindness in adults[1]. Worldwide, the number of adults with diabetic retinopathy was estimated to be 103.12 million in 2020 and is expected to increase to 160.50 million by 2045[2]. In China, 18.45% of patients with diabetes mellitus have diabetic retinopathy[3], and the independent risk factors for diabetic retinopathy are younger age, higher systolic blood pressure (SBP), longer duration of diabetes mellitus, and poor glycemic control[4]. However, despite these traditional indicators of risk, wide variations in the development and severity of diabetic retinopathy exist which cannot be completely explained by these known factors[5]. Therefore, diabetic retinopathy risk factors are not fully understood.

Dehydroepiandrosterone (DHEA), an androgen precursor, is an abundant steroid hormone in human circulation. Previous studies have reported that DHEA improves endothelial cell function, inhibits inflammation, and reverses vascular

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remodeling[6,7]. Low DHEA levels are associated with the risk of coronary heart disease (CHD) in the general population and individuals with type 2 diabetes mellitus[8,9]. Furthermore, our previous study demonstrated an inverse relationship between low serum DHEA and diabetic kidney disease (DKD) in men with type 2 diabetes mellitus[10]. In recent years, the point of a unifying mechanism has been raised in the pathogenesis of micro- and macrovascular complications of diabetes mellitus. These common pathogenic pathways included the production of reactive oxygen species, oxidative stress, and chronic low-grade inflammation[11]. In animal experiments, DHEA attenuated the adverse effects of hyperglycemia on bovine retinal pericytes[12]. However, the association of DHEA with the risk of diabetic retinopathy remains unclear in patients with diabetes mellitus.

Therefore, this cross-sectional study assessed the association of DHEA with the risk of diabetic retinopathy in patients with type 2 diabetes mellitus in China.

MATERIALS AND METHODS

Study design

This cross-sectional study was conducted at the Department of Endocrinology and Metabolism, Tianjin Medical University General Hospital in Tianjin, China. Hospitalized patients with type 2 diabetes mellitus were enrolled and DHEA measured between October 12, 2020, and June 30, 2022. The patients were admitted for glycemic control and evaluation of diabetic complications. The type of diabetes mellitus was determined by physicians based on clinical features, including the onset age, acute or chronic onset, body mass index (BMI), fasting or post glucose-challenge insulin and C-peptide levels, insulin dependence, and pancreatic beta cell autoantibodies if necessary. If multiple medical records for one patient existed, only one of the patient's records was included. The exclusion criteria were: age <18 years, pregnancy, use of glucocorticoids or sex hormones, polycystic ovarian syndrome, hypopituitarism, and adrenal disease, including primary hyperaldosteronism, Cushing's syndrome, subclinical Cushing's syndrome, pheochromocytoma, and adrenal insufficiency. Moreover, participants without fundus photograph information were excluded. None of the participants enrolled in this study used DHEA. Figure 1 details the study population identification process.

The Institutional Review Board of Tianjin Medical University General Hospital approved this study (approval number: IRB2020-YX-027-01) and waived the informed consent requirement because the data were gathered from electronic medical records, and the participants' identities were anonymized.

Data collection

Clinical data for each participant were extracted from electronic medical records, including age, sex, height, body weight, medical insurance type, smoking and drinking status, medical history (type 2 diabetes mellitus, hypertension, and cardiovascular disease [CVD]), SBP, diastolic blood pressure (DBP), lipid profiles, fasting blood glucose (FBG) level, glycosylated hemoglobin (HbA1c) level, uric acid concentration, creatinine level, the urine albumin to creatinine ratio (ACR), and medication use (hypotensive, lipid-lowering, and glucose-lowering medications). The BMI was calculated as the participant's weight (kg) divided by height (meters) squared. Obesity was defined as a BMI >27.5 kg/m^2 [13]. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation[14].

The serum DHEA concentration was quantified using liquid chromatography–tandem mass spectrometry as described previously[10,15]. Briefly, fasting blood samples were collected in the morning after admission and immediately sent to the Laboratory of Endocrinology and Metabolism at the Tianjin Medical University General Hospital. Two professionals pre-treated the samples following uniform standards and then loaded them into a Jasper™ HPLC system coupled to an AB SCIEX Triple Quad™ 4500MD mass spectrometer (AB SCIEX, Framingham, MA, USA) to measure DHEA.

Definitions

Diabetes mellitus was defined as a fasting blood glucose level ≥7.0 mmol/L, a 2 hour plasma glucose level ≥11.1 mmol/L, an HbA1c level ≥6.5%, a self-reported history of diabetes mellitus, or the use of hypoglycemic medications[16]. Hypertension was defined as an SBP ≥140 mmHg, a DBP ≥90 mmHg, a self-reported history of hypertension, or the use of hypotensive medications[17]. Dyslipidemia was defined as a total cholesterol (TC) level ≥6.2 mmol/L, a triglyceride (TG) level ≥2.3 mmol/L, a low-density lipoprotein cholesterol (LDL-C) level ≥4.1 mmol/L, a high-density lipoprotein cholesterol (HDL-C) level <1.0 mmol/L, or the use of lipid-lowering medications[18]. Diabetic kidney disease was defined as an albumin to creatinine ratio value >30 mg/g or an eGFR level <60 mL/min/1.73 m^2 [19].

Experienced and trained specialists took standard non-mydriatic fundus photographs to evaluate diabetic retinopathy. Diabetic retinopathy was diagnosed based on microaneurysms, hard exudates, cotton wool spots, intraretinal hemorrhages, venous beading changes, intraretinal microvascular anomalies, neovascularization, vitreous hemorrhage, or tractional retinal detachment[20]. The International Classification of Diabetic Retinopathy Scale[20] defines sight-threatening diabetic retinopathy as severe non-proliferative diabetic retinopathy (NPDR), proliferative diabetic retinopathy (PDR) or diabetic macular edema. Thus, the patients were divided into non-diabetic retinopathy, non-sight-threatening diabetic retinopathy (i.e., mild or moderate NPDR), and sight-threatening diabetic retinopathy (i.e., severe NPDR, PDR or diabetic macular edema) groups.

Statistical analyses

Normally distributed continuous variables were presented as mean ± standard deviation, and between-group comparisons were performed using Student's *t*-tests or one-way analysis of

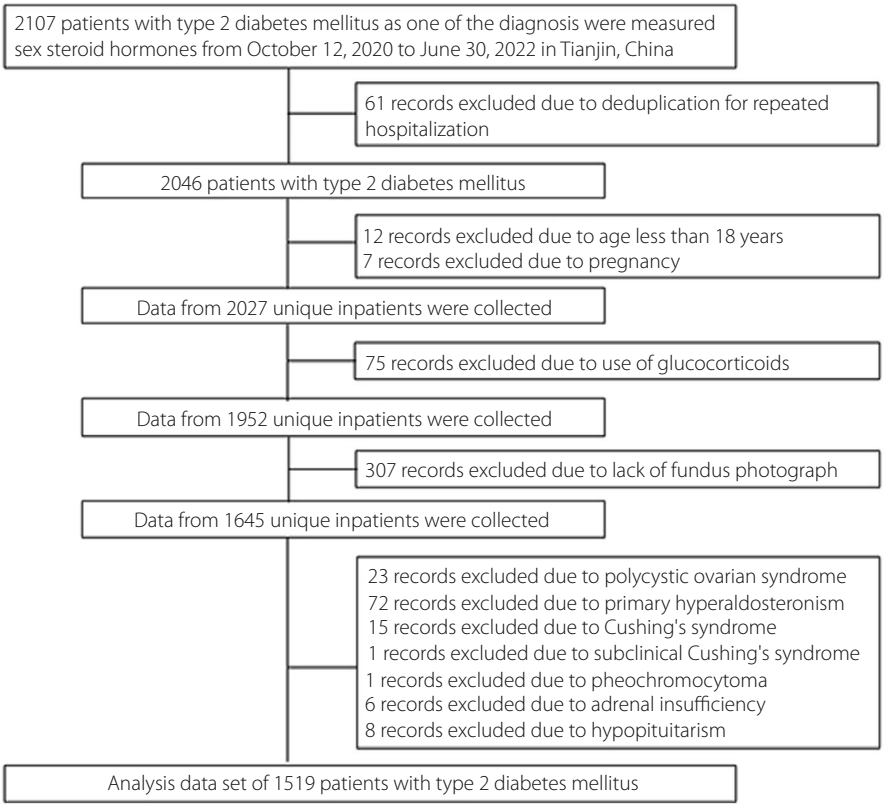

Figure 1 | Flow chart of the identification of the study population. There were 1,519 patients with type 2 diabetes mellitus involved in the final analysis.

variance. Non-normally distributed variables were expressed as medians with interquartile ranges, and between-group comparisons were conducted using Mann–Whitney *U* or Kruskal–Wallis tests. Categorical variables were described as numbers with percentages, and between-group comparisons were performed using the chi-squared tests.

Multivariate logistic regression analyses were used to evaluate the association of DHEA with diabetic retinopathy after adjusting for confounding factors. Potential confounders were determined according to univariate findings (including the insurance type, BMI, duration of diabetes mellitus, SBP, DBP, and diabetic kidney disease [yes/no], the HDL-C, FBG, and HbA1c levels, the use of metformin, α-glucosidase inhibitors, and insulin [all $P < 0.05$]) and literature reports (including age)[4,21]. The serum DHEA levels were equally categorized into quartiles, and the lowest quartile was used as a reference. The restricted cubic spline with four knots (5th, 35th, 65th, and 95th percentiles) was used to model the association of DHEA with diabetic retinopathy and to depict the overall dose–response correlation.

Moreover, subgroup analyses were performed to determine the relationship between DHEA and diabetic retinopathy based on the following subgroups: age (<65 or ≥65 years), sex, obesity status, hypertension, dyslipidemia, and HbA1c (<7.0 or ≥7.0%). An interaction test in the logistic regression analyses was used to compare the effects of DHEA on diabetic retinopathy between the analyzed subgroups. The DHEA data were log-transformed with the base natural constant in the logistic regression analyses and restricted cubic spline. A two-sided *P*-value less than 0.05 was considered statistically significant. Analyses were performed using SPSS for Windows (version 25.0; Armonk, NY, USA) and R software (version 4.1.3; R Foundation, Vienna, Austria).

RESULTS

Clinical characteristics of participants

Table 1 presents the clinical characteristics of the participants based on the DHEA quartiles. During the study period, 2,107 patients with type 2 diabetes mellitus were hospitalized, and 1,519 participants were included in the analysis; 826 (54.4%) were men, and the mean overall age was 55.60 ± 14.17 years. The prevalences of non-sight-threatening diabetic retinopathy and sight-threatening diabetic retinopathy were 20.3% and 2.4%, respectively. The median duration of diabetes mellitus was 7.00 (1.00–15.00) years. The mean fasting blood glucose and HbA1c levels were 7.77 ± 2.89 mmol/L and 8.68 ± 2.21%, respectively. Participants in the higher quartiles were younger, had a shorter duration of diabetes mellitus, and had fewer instances of diabetic kidney disease, cardiovascular disease, hypertension, dyslipidemia, and medication use (hypotensive and lipid-lowering medications, sulfonylureas, metformin, α-glucosidase inhibitors, dipeptidyl peptidase 4 inhibitors, and

Table 1 | Characteristics of patients categorized by quartiles of DHEA level

Variables	Overall	Quartiles of DHEA level				*P*
		Quartile 1	Quartile 2	Quartile 3	Quartile 4	
Participants	1,519	380	308	379	380	–
Age, years	55.60 ± 14.17	63.14 ± 11.32	58.57 ± 12.26	53.69 ± 13.41	46.99 ± 14.25	<0.001
Male sex, %	826 (54.4)	196 (51.6)	230 (60.5)	201 (53.0)	199 (52.4)	0.048
BMI, kg/m^2	27.15 ± 5.46	26.33 ± 4.36	26.79 ± 4.96	27.39 ± 6.16	28.04 ± 6.00	<0.001
Current smoking, %	432 (28.5)	88 (23.3)	126 (33.2)	117 (31.0)	101 (26.6)	0.012
Current drinking, %	407 (26.9)	83 (22.0)	113 (29.7)	105 (27.8)	106 (28.0)	0.084
Insurance type, %						
Urban workers	1,262 (83.1)	310 (81.6)	330 (86.8)	325 (85.8)	297 (78.2)	0.013
Non-working urban residents	179 (11.8)	48 (12.6)	36 (9.5)	42 (11.1)	53 (13.9)	
Self-pay	78 (5.1)	22 (5.8)	14 (3.7)	12 (3.2)	30 (7.9)	
Duration of type 2 diabetes, year	7.00 (1.00, 15.00)	10.00 (3.00, 20.00)	10.00 (2.00, 17.00)	7.00 (1.00, 13.00)	3.00 (0.16, 10.00)	<0.001
DR status, %						
Non-DR	1,173 (77.2)	267 (70.3)	297 (78.2)	291 (76.8)	318 (83.7)	0.002
Non-sight-threatening DR	309 (20.3)	100 (26.3)	74 (19.5)	77 (20.3)	58 (15.3)	
Sight-threatening DR	37 (2.4)	13 (3.4)	9 (2.4)	11 (2.9)	4 (1.1)	
DKD, %	447 (31.9)	157 (44.0)	120 (33.7)	84 (23.9)	86 (25.6)	<0.001
CVD, %	334 (22.0)	130 (34.2)	96 (25.3)	70 (18.5)	38 (10.0)	<0.001
Hypertension, %	911 (60.0)	259 (68.2)	254 (66.8)	213 (56.2)	185 (48.7)	<0.001
Dyslipidemia, %	1,122 (74.7)	310 (82.4)	270 (72.4)	282 (75.0)	260 (68.8)	<0.001
Use of hypotensive medications, %	759 (50.2)	238 (62.8)	217 (57.4)	173 (46.0)	131 (34.6)	<0.001
Use of lipid-lowering medications, %	389 (25.7)	145 (38.3)	101 (26.6)	77 (20.4)	66 (17.5)	<0.001
Glucose-lowering medications						
Sulfonylureas, %	195 (13.1)	67 (18.3)	49 (13.1)	51 (13.7)	28 (7.4)	<0.001
Glinides, %	95 (6.4)	29 (7.9)	28 (7.5)	16 (4.3)	22 (5.8)	0.164
Metformin, %	575 (38.6)	155 (42.3)	160 (42.8)	141 (37.9)	119 (31.6)	0.005
Thiazolidinediones, %	32 (2.1)	9 (2.5)	12 (3.2)	7 (1.9)	4 (1.1)	0.220
α-glucosidase inhibitors, %	504 (33.8)	161 (44.0)	140 (37.4)	116 (31.2)	87 (23.1)	<0.001
DPP-4 inhibitors, %	192 (12.9)	55 (15.0)	57 (15.2)	45 (12.1)	35 (9.3)	0.048
GLP-1 receptor agonists, %	97 (6.5)	23 (6.3)	16 (4.3)	34 (9.1)	24 (6.4)	0.062
SGLT-2 inhibitors, %	167 (11.2)	47 (12.8)	42 (11.2)	45 (12.1)	33 (8.8)	0.313
Insulin, %	513 (34.5)	157 (42.9)	146 (39.0)	114 (30.6)	96 (25.5)	<0.001
Blood pressure, mmHg						
Systolic	136.59 ± 18.07	137.14 ± 19.32	137.13 ± 17.55	136.04 ± 18.17	136.04 ± 17.19	0.708
Diastolic	83.11 ± 11.92	81.02 ± 11.47	82.23 ± 11.70	83.66 ± 11.33	85.53 ± 12.70	<0.001
TC, mmol/L	4.98 ± 1.57	4.71 ± 1.32	4.84 ± 1.24	5.19 ± 1.72	5.18 ± 1.84	<0.001
TG, mmol/L	1.75 (1.27, 2.54)	1.62 (1.18, 2.26)	1.78 (1.27, 2.40)	1.83 (1.32, 2.72)	1.83 (1.29, 3.00)	0.001
HDL-C, mmol/L	1.08 ± 0.26	1.06 ± 0.28	1.06 ± 0.25	1.09 ± 0.26	1.10 ± 0.26	0.065
LDL-C, mmol/L	3.01 ± 0.98	2.81 ± 1.02	2.95 ± 0.92	3.10 ± 0.95	3.15 ± 1.00	<0.001
FBG, mmol/L	7.77 ± 2.89	7.49 ± 2.87	7.66 ± 2.80	7.85 ± 3.06	8.08 ± 2.79	0.038
HbA1c, %	8.68 ± 2.21	8.71 ± 2.34	8.72 ± 2.22	8.62 ± 2.17	8.67 ± 2.11	0.939
Uric acid, μmol/L	342.38 ± 104.00	343.80 ± 108.65	345.13 ± 101.88	342.14 ± 104.15	338.43 ± 101.46	0.833
eGFR, mL/(min*1.73 m^2)	105.82 ± 24.15	95.11 ± 24.69	100.31 ± 24.53	110.31 ± 20.88	117.66 ± 19.58	<0.001
ACR, mg/g	15.00 (7.30, 39.65)	20.20 (9.78, 90.17)	15.02 (7.83, 47.38)	12.85 (7.00, 28.53)	13.30 (6.40, 29.55)	<0.001

ACR, albumin to creatinine ratio; BMI, body mass index; CVD, cardiovascular disease; DHEA, dehydroepiandrosterone; DKD, diabetic kidney disease; DPP-4, dipeptidyl peptidase-4; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; GLP-1, glucagon-like peptide-1; HbA1c, glycosylated hemoglobin; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; SGLT-2, sodium-glucose cotransporter-2; TC, total cholesterol; TG, triglycerides.

insulin) than those in the lower quartiles (all $P < 0.05$). Furthermore, BMI, DBP, eGFR, and the TC, TG, LDL-C, FBG levels significantly trended upward, whereas the ACR significantly trended downward (all $P < 0.05$).

Table 2 provides the clinical characteristics of the study population based on the diabetic retinopathy status. Overall, 346 of 1,519 patients (22.8%) had diabetic retinopathy. Those with diabetic retinopathy had a longer duration of diabetes mellitus,

Table 2 | Characteristics of patients categorized by the presence of diabetic retinopathy

Variables	Non-DR	DR	*P*
Participants, %	1,173 (77.2)	346 (22.8)	–
Age, years	55.25 ± 14.53	56.77 ± 12.86	0.062
Male sex, %	648 (55.2)	178 (51.4)	0.213
BMI, kg/m^2	27.42 ± 5.62	26.22 ± 4.75	<0.001
Current smoking, %	343 (29.3)	89 (25.9)	0.214
Current drinking, %	325 (27.8)	82 (23.8)	0.147
Insurance type, %			
Urban workers	989 (84.3)	273 (78.9)	0.025
Non-working urban residents	124 (10.6)	55 (15.9)	
Self-pay	60 (5.1)	18 (5.2)	
Duration of type 2 diabetes, year	6.00 (0.50, 13.00)	10.00 (4.00, 19.00)	<0.001
DKD, %	286 (26.4)	161 (50.5)	<0.001
CVD, %	251 (21.4)	83 (24.0)	0.310
Hypertension, %	687 (58.6)	224 (64.7)	0.039
Dyslipidemia, %	877 (75.5)	245 (71.8)	0.176
Use of hypotensive medications, %	579 (49.5)	180 (52.6)	0.306
Use of lipid-lowering medications, %	301 (25.7)	88 (25.6)	0.957
Glucose-lowering medications			
Sulfonylureas, %	140 (12.2)	55 (16.2)	0.052
Glinides, %	73 (6.3)	22 (6.5)	0.925
Metformin, %	423 (36.8)	152 (44.8)	0.007
Thiazolidinediones, %	26 (2.3)	6 (1.8)	0.584
α-Glucosidase inhibitors, %	357 (31.0)	147 (43.4)	<0.001
DPP-4 inhibitors, %	138 (12.0)	54 (15.9)	0.058
GLP-1 receptor agonists, %	76 (6.6)	21 (6.2)	0.786
SGLT-2 inhibitors, %	129 (11.2)	38 (11.2)	0.997
Insulin, %	336 (29.2)	177 (52.2)	<0.001
Blood pressure, mmHg			
Systolic	135.63 ± 17.26	139.84 ± 20.26	0.001
Diastolic	82.74 ± 11.45	84.36 ± 13.32	0.041
TC, mmol/L	4.95 ± 1.60	5.07 ± 1.43	0.221
TG, mmol/L	1.76 (1.27, 2.56)	1.69 (1.25, 2.48)	0.141
HDL-C, mmol/L	1.06 ± 0.26	1.12 ± 0.27	<0.001
LDL-C, mmol/L	2.99 ± 0.98	3.07 ± 1.00	0.189
FBG, mmol/L	7.66 ± 2.76	8.15 ± 3.23	0.012
HbA1c, %	8.57 ± 2.20	9.07 ± 2.21	<0.001
Uric acid, μmol/L	343.12 ± 103.97	339.92 ± 104.22	0.618
eGFR, mL/(min*1.73 m^2)	107.30 ± 23.19	100.82 ± 26.56	<0.001
ACR, mg/g	13.50 (6.99, 29.28)	28.05 (9.43, 170.58)	<0.001

ACR, albumin to creatinine ratio; BMI, body mass index; CVD, cardiovascular disease; DKD, diabetic kidney disease; DPP-4, dipeptidyl peptidase-4; DR, diabetic retinopathy; eGFR, estimated glomerular filtration rate; FBG, fasting blood glucose; GLP-1, glucagon-like peptide-1; HbA1c, glycosylated hemoglobin; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; SGLT-2, sodium-glucose cotransporter-2; TC, total cholesterol; TG, triglycerides.

more instances of diabetic kidney disease and hypertension, and higher SBP, DBP, ACR, HDL-C, FBG, and HbA1c, but lower BMI and eGFR values than those without diabetic retinopathy (all $P < 0.05$). Furthermore, patients with diabetic retinopathy were more likely to use metformin, α-glucosidase inhibitors, and insulin than those without diabetic retinopathy (all $P < 0.05$). Finally, the proportion of medical insurance type significantly differed between patients with and without diabetic retinopathy ($P = 0.025$).

DHEA associations with diabetic retinopathy

Table 3 presents the odds ratios (ORs) for the association of DHEA with diabetic retinopathy in three models. The diabetic retinopathy odds decreased significantly as the DHEA level increased incrementally in model 1 (unadjusted; OR [quartile 4 compared with quartile 1]: 0.46; 95% confidence interval [CI]: 0.33–0.65; $P < 0.001$ for the trend). Furthermore, a low serum DHEA level remained statistically associated with diabetic retinopathy after adjusting for age, insurance type, BMI, duration

Table 3 | Odds ratios of diabetic retinopathy by different status of DHEA

	No. of participants	No. of cases	Model 1		Model 2		Model 3	
			OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
Quartile 1	380	113	Reference	–	Reference	–	Reference	–
Quartile 2	380	83	0.66 (0.48, 0.92)	0.013	0.68 (0.49, 0.95)	0.022	0.56 (0.37, 0.85)	0.006
Quartile 3	379	88	0.72 (0.52, 0.99)	0.042	0.74 (0.52, 1.03)	0.076	0.70 (0.46, 1.07)	0.102
Quartile 4	380	62	0.46 (0.33, 0.65)	<0.001	0.47 (0.32, 0.69)	<0.001	0.51 (0.32, 0.81)	0.005
P for trend				<0.001		0.001		0.012
As a continuous variable[†]	1,519	346	0.66 (0.54, 0.81)	<0.001	0.67 (0.53, 0.83)	<0.001	0.71 (0.54, 0.94)	0.015

[†]DHEA was log-transformed with base natural constant. Model 1: unadjusted. Model 2: adjusts for age and insurance type. Model 3: model 2 + BMI, duration of diabetes mellitus, SBP, DBP, DKD, HDL-C, FBG, HbA1c, and use of metformin, α-glucosidase inhibitors, and insulin. BMI, body mass index; CI, confidence interval; DBP, diastolic blood pressure; DHEA, dehydroepiandrosterone; DKD, diabetic kidney disease; DR, diabetic retinopathy; FBG, fasting blood glucose; HbA1c, glycosylated hemoglobin; HDL-C, high density lipoprotein cholesterol; OR, odds ratio; SBP, systolic blood pressure.

of diabetes mellitus, SBP, DBP, diabetic kidney disease, HDL-C, FBG, HbA1c, and the use of metformin, α-glucosidase inhibitors, and insulin in model 3 (OR: 0.51, 95% CI: 0.32–0.81; $P = 0.012$ for the trend). Moreover, when DHEA was log-transformed with the base natural constant and analyzed as a continuous variable, a significant association between DHEA and diabetic retinopathy risk remained after adjusting for the abovementioned variables (OR: 0.71; 95% CI: 0.54–0.94; $P = 0.015$).

Figure 2 describes the overall dose–response association of DHEA with diabetic retinopathy in the restricted cubic spline. After adjusting for covariates, the odds of diabetic retinopathy decreased linearly with increasing DHEA concentrations (*P*-overall = 0.044; *P*-nonlinear = 0.364).

Subgroup analyses of the DHEA and DR relationship

Figure 3 illustrates the association between DHEA and diabetic retinopathy in the subgroup analyses based on age, sex, obesity, hypertension, dyslipidemia, and the HbA1c level. The effect of DHEA on diabetic retinopathy risk was stable in all subgroups, and no interactions between DHEA and the subgroup variables were statistically significant (all *P* for interaction >0.05).

DISCUSSION

This cross-sectional study assessed the association of DHEA levels with diabetic retinopathy risk in patients with type 2 diabetes mellitus. To our knowledge, we are the first to report a significant association between low serum DHEA levels and DR in type 2 diabetes mellitus patients after adjusting for

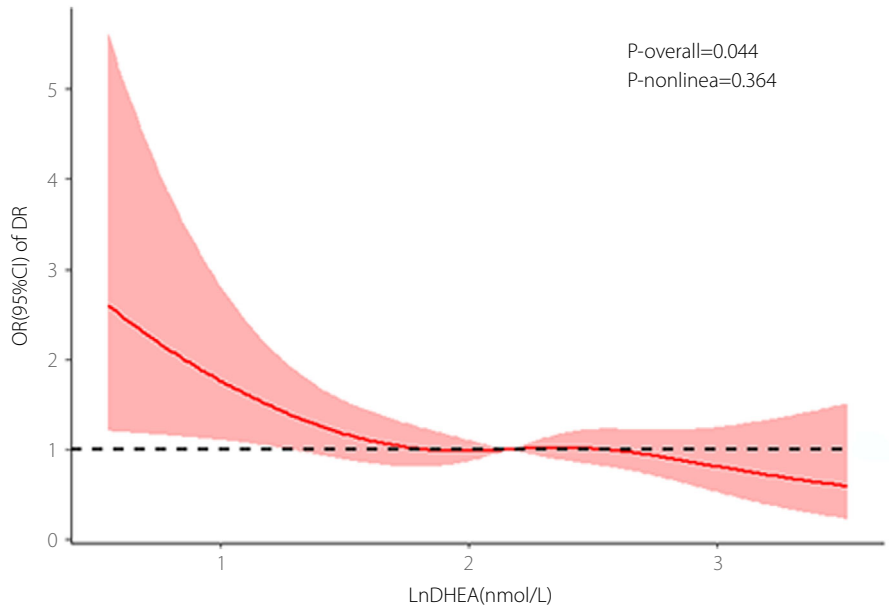

Figure 2 | The overall dose–response association of dehydroepiandrosterone (DHEA) with diabetic retinopathy (DR) shown by the restricted cubic spline. The line indicated the adjusted ORs and 95% CI is shown by shaded areas. DHEA was log-transformed with base natural constant. Adjusted for age, insurance type, BMI, duration of diabetes, SBP, DBP, DKD, HDL-C, FBG, HbA1c, and use of metformin, α-glucosidase inhibitors and insulin.

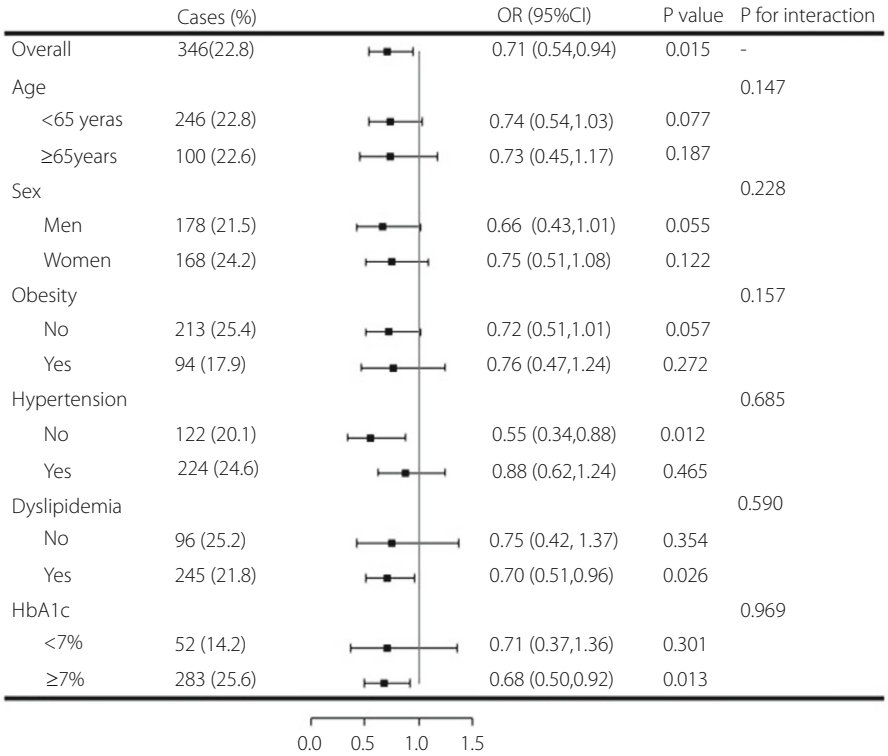

Figure 3 | Subgroup analyses of the association of DHEA with diabetic retinopathy. DHEA was log-transformed with base natural constant. Adjusted for age, insurance type, BMI, duration of diabetes mellitus, SBP, DBP, DKD, HDL-C, FBG, HbA1c, and use of metformin, α-glucosidase inhibitors and insulin.

traditional risk factors. Moreover, the effect of DHEA on diabetic retinopathy was stable in the age, sex, obesity, hypertension, dyslipidemia, and HbA1c subgroups.

Previous studies have evaluated associations of DHEA with macrovascular and microvascular diseases. In men, a low DHEA concentration was associated with an increased incidence of macrovascular diseases. A prospective study of the general population demonstrated that a low serum DHEA level was associated with a 5 year risk of developing coronary heart disease in men aged 69–81 years[8]. The Massachusetts Male Aging Study also reported a significant association between low serum DHEA levels and the development of ischemic heart disease[22]. Moreover, low DHEA levels correlated with cardiovascular disease, including coronary heart disease, myocardial infarction, and stroke, in participants with type 2 diabetes mellitus from 10 communities in Shanghai, China[9]. Similarly, our previous study identified a relationship between low DHEA levels and coronary heart disease in men with type 2 diabetes mellitus[15]. Conversely, a significant relationship was not identified between DHEA and the incidence of cardiovascular disease in studies of women in the general population or those with type 2 diabetes mellitus[9,15,23]. Sex-specific differences regarding these associations remain unclear and require further investigation.

Previous studies have described the relationship between DHEA and microvascular disease. The population-based Rotterdam study showed that DHEA was not related to microvascular injury in men or women, evaluated by arteriolar and venular calibers of the retina[24]. However, a study of postmenopausal women with type 2 diabetes mellitus from Shanghai, China, reported that high DHEA levels were associated with diabetic kidney disease[9]. In contrast, a rat mesangial cell-based study showed that DHEA had a protective effect against hyperglycemia-induced lipid peroxidation and cell growth inhibition[25]. Our previous study also reported that low serum DHEA levels correlated with diabetic kidney disease in men with type 2 diabetes mellitus[10]. In addition, DHEA was shown to reverse bovine retinal capillary pericyte loss caused by glucose toxicity[12]. Similarly, the current study identified a strong negative correlation between the serum DHEA level and diabetic retinopathy in participants with type 2 diabetes mellitus. We presume that differences in the participants, outcomes, and adjusted risk factors partially explain these contrasting results.

Presently, diabetic retinopathy is considered an inflammatory neurovascular complication of diabetes mellitus and is characterized by retinal capillary occlusion, vasculature leakage, retinal ischemia and damage, angiogenesis, and neovascularization[26]. Inflammation plays an important role in the pathogenesis of diabetic retinopathy[27], and increases in endothelial cell adhesion molecules, such as VCAM-1 and E-selectin, cause an accumulation of leukocytes in retinal capillaries[28–31]. Leukocyte-

endothelium adhesion leads to endothelial cell apoptosis and the breakdown of the blood–retinal barrier[32], thereby causing a low-grade inflammatory state in the retina. In addition, the upregulated inflammatory cytokine expression in the serum and ocular samples of diabetic patients, including TNF-α, IL-6, IL-8, and soluble VCAM-1, have been associated with the severity of diabetic retinopathy[33–35].

Furthermore, DHEA inhibits inflammation and regulates immune responses. For example, in a lipopolysaccharide (LPS)-induced lung inflammation model, DHEA restrained acute neutrophil recruitment by upregulating developmental endothelial locus 1 expression, which is decreased in an inflammatory state[36]. In mice with colitis, DHEA attenuates intestinal inflammatory injury through GPR30-mediated Nrf2 activation and NLRP3 inflammasome inhibition[37]. Moreover, DHEA relieves *Escherichia coli* O157:H7-induced inflammation in mice and LPS-induced inflammation in RAW264.7 macrophages by blocking the activation of AKT, MAPK, and NF-κB signaling pathways and increasing the activation of Nrf2, which is associated with autophagy[38,39]. Given that diabetic retinopathy is also an inflammatory complication of diabetes mellitus, we hypothesized that the anti-inflammatory effects of DHEA partly explain the inverse relationship between DHEA and diabetic retinopathy.

Neurodegeneration is an important part in the pathogenesis of diabetic retinopathy[40]. Neurodegeneration, including reactive gliosis, decreased retinal neuronal function, and neuronal apoptosis, is now regarded as an early event in the progression of diabetic retinopathy[41,42]. Diabetes mellitus-induced neurodegeneration occurs before visible microangiopathy in diabetic rats and humans[43,44]. Moreover, DHEA has neuroprotective effects on stroke and traumatic brain and spinal injuries[45–47]. Furthermore, DHEA inhibits microglial inflammation by activating the TrkA-Akt1/2-CREB-Jmjd3 pathway in subarachnoid hemorrhage and neuroinflammation models[48,49]. Additionally, DHEA prevents the apoptotic loss of neurons through its interaction with nerve growth factor[50], and intravitreal DHEA injections reduces retinal damage caused by the excitatory amino acid, AMPA, in adult Sprague–Dawley rats[51]. Furthermore, BNN27, a novel C17-spiroepoxide derivative of DHEA, reverses retinal injury in diabetic rats, targeting the neurodegenerative and inflammatory components of diabetic retinopathy[52]. Therefore, we speculate that the neuroprotective mechanisms of DHEA underlie the relationship between DHEA and diabetic retinopathy.

Although animal experiments have presented favorable evidence regarding the effects of DHEA on inflammation and neurodegeneration, DHEA supplementation in humans has been controversial since these studies only included a small number of participants. For instance, a daily 50 mg dose of DHEA significantly increased insulin sensitivity[53] and decreased the total cholesterol and low-density lipoprotein levels[54,55] in patients with hypoadrenalism and healthy postmenopausal women. Furthermore, 50 mg of DHEA per day for 6 months improved age-related changes in fat mass, lean mass, and bone mineral density in older women and men[56,57]. However, a systematic review and meta-analysis of randomized controlled trials indicated that DHEA administration reduced fasting plasma glucose levels but not insulin resistance[58]. In contrast, other studies have demonstrated that DHEA treatment does not affect insulin secretion[59] or lipid profiles[60]. DHEA supplementation did not affect cardiovascular parameters, arterial stiffness, or endothelial function in women with hypoadrenalism or hypopituitarism[61,62]. Thus, further clinical trials with larger sample sizes are needed to confirm the effect of DHEA on glucose metabolism and health.

This study had several limitations. First, the causal association of DHEA with diabetic retinopathy could not be determined because of the study's cross-sectional design. Second, we recruited hospitalized patients with type 2 diabetes mellitus; thus, these results should be confirmed before generalizing them to the general diabetic population. Third, we did not assess the association between low DHEA levels and the severity of diabetic retinopathy, especially sight-threatening diabetic retinopathy because of the small number of patients with severe diabetic retinopathy. Finally, this study's sample size was relatively small, potentially limiting the power to test subgroup interactions.

In conclusion, low serum DHEA levels were significantly associated with diabetic retinopathy in adult patients with type 2 diabetes mellitus in northern China, suggesting a potential role of DHEA in the pathogenesis of diabetic retinopathy. Further prospective studies are necessary to confirm these results and to identify the mechanisms underlying associations between DHEA and diabetic microvascular complications.

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DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: The study protocol was approved by the institutional review board of Tianjin Medical University General Hospital (approval number: IRB2020-YX-027-01).

Informed consent: The requirement for informed consent was waived because the data were gathered from electronic medical records, and the participants' identities were anonymized.

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