CLINICAL TRIAL

Normal parathyroid hormone and non-proliferative diabetic retinopathy in patients with type 2 diabetes

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ABSTRACT

Aims/Introduction: To investigate the associations between parathyroid hormone (PTH) and non-proliferative diabetic retinopathy (NPDR) in patients with type 2 diabetes mellitus.

Materials and Methods: Data were collected from 2,322 patients with type 2 diabetes mellitus in hospital between 2017 and 2019. The odds ratio (OR) and the corresponding 95% confidence interval related to the quartiles of PTH were obtained by logistic regression analysis after adjusting the potential confounding variation.

Results: The patients were stratified into quartiles (Q1–Q4) based on the PTH levels, with the cut-off limits of ≤23.74, 23.74–29.47, 29.47–37.30 and >37.30 pg/mL in men, and ≤24.47, 24.47–31.22, 31.22–39.49 and >39.49 pg/mL in women. The first quartile (Q1) represents the lowest quartile and the fourth quartile (Q4) is the highest. According to the quartiles (Q1–Q4), the prevalence rate of NPDR in patients showed a significantly decreasing trend (37.9%, 36.3%, 34.0% vs 24.0% in men; 43.2%, 40.5%, 31.1% vs 26.2% in women, both $P < 0.05$). Independent of age, diabetes duration and other metabolic factors, multivariate logistic regression showed that participants in Q4 had a lower OR of NPDR than those in Q1 (OR 0.443, 95% confidence interval 0.300–0.654, $P < 0.001$ for men; OR 0.428, 95% confidence interval 0.283–0.646, $P < 0.001$ for women).

Conclusions: Low serum PTH levels were significantly associated with complications of NPDR in inpatients. Its causality remains to be further studied.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disease with rapidly increasing prevalence worldwide. Diabetic retinopathy (DR) is one of the most common microvascular complications of type 2 diabetes mellitus, and is a leading cause of irreversible blindness among adults aged 20–74 years[1]. Strict control of blood pressure and blood glucose are vital management strategies to prevent the progression of DR. Although laser photocoagulation and therapeutic vascular endothelial growth factor antibody are effective treatments[2], the prevalence of DR is still high, thus early identification and intervention are very important. Identifying biomarkers related to DR and exploring retinal

targeted intervention methods will help to delay the progression of DR.

Parathyroid hormone (PTH) is a systemic hormone that is known to regulate calcium and phosphate homeostasis by promoting bone resorption, suppressing urinary calcium loss and accelerating the activation of vitamin D. Epidemiological studies have suggested that patients with diabetes mellitus or IGT are more likely to suffer from parathyroid dysfunction than the general population[3,4]. It means that diabetes mellitus can cause disorders of endocrine factors related to mineral metabolism[5]. For instance, the progression of diabetic nephropathy is related to the level of PTH and serum phosphate[6]. Low circulating concentrations of 25-hydroxyvitamin D and high circulating calcium are associated with an increased risk of macrovascular and microvascular disease events in type 2 diabetes mellitus[7–10]. In a large population-based study, elevated PTH levels were

independently associated with diabetes mellitus risk in white people[11]. Controversially, a recently published Mendelian randomization study proved that genetically predicted serum calcium and PTH levels were not related to type 2 diabetes mellitus[12]. The relationship between parathyroid function and type 2 diabetes mellitus has been a research focus for concern, and this relationship has extended to the field of normal parathyroid function. Recent cohort studies showed that elevated serum phosphate concentration within the normal range was associated with microvascular dysfunction in participants[13,14]. However, there are scarce data on the relationship between normal PTH levels and DR. Therefore, the role of normal PTH levels in DR requires further research. Thus, in a retrospective cross-sectional study, we investigated the potential association between normal PTH levels and non-proliferative diabetic retinopathy (NPDR) risk in men and women with diabetes.

METHODS

Study population

We set up a database of type 2 diabetes mellitus inpatients at Affiliated Hospital of Medical College Qingdao University (Shandong, China). Analyzed data were collected from the database between 2017 to 2019. The inclusion criteria were in accordance with the American Diabetic Association 2014 criteria[15]: glycated hemoglobin (HbA1c) $\geq$6.5%, or fasting plasma glucose $\geq$126 mg/dL (7.0 mmol/L), or 2-h plasma glucose $\geq$200 mg/dL (11.1 mmol/L) during an oral glucose tolerance test, or a random plasma glucose $\geq$200 mg/dL (11.1 mmol/L) with classic symptoms of hyperglycemia or hyperglycemic crisis. The Participants were excluded if they were aged <18 years or >80 years, had acute complications of type 2 diabetes mellitus, abnormal PTH level, parathyroid disease history, osteoporosis, severe heart failure, severe liver disease, renal insufficiency requiring dialysis and malignant tumors or were taking drugs that affect parathyroid function. Pregnant or breast-feeding women were also excluded (Figure 1).

The protocol was designed in accordance with the Helsinki Declaration and approved by the Ethics Committee of the Affiliated Hospital of Medical College Qingdao University. All participants provided written informed consent. The study is registered on http://www.chictr.org.cn/ under the registration number ChiCTR2000032374.

Data collection

Anthropometric parameters of patients included age, height, weight, diabetes duration, the status of drinking and smoking, and blood pressure. Through laboratory examination, we measured the following indicators: fasting plasma glucose, HbA1c, PTH, thyroid-stimulating hormone, free triiodothyronine, free thyroid hormone, serum electrolyte, 25-hydroxyvitamin D_3, low-density lipoprotein-cholesterol, high-density lipoprotein-cholesterol, free fatty acid, lipid profiles including triglycerides, total cholesterol, serum creatinine and serum uric acid (sUA).
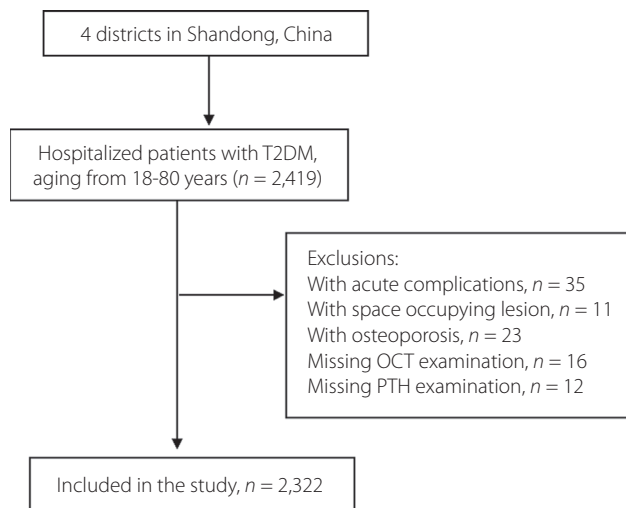
Figure 1 | Flowchart of the inclusion and exclusion of participants. OCT, optical coherence tomography; PTH, parathyroid hormone; T2DM, type 2 diabetes mellitus.

Blood pressure (BP) was measured after a 5-min rest, and averaged for two or more consecutive days. Body mass index (BMI) was calculated as the weight in kilograms divided by height in meters squared. After fasting for at least 8 h, the blood sample is pierced into the median elbow vein, centrifuged within 1 h, stored in the cold chain within 2–4 h, and transported to the central laboratory for testing. The determination of serum PTH adopts electrochemiluminescence immunoassay, and its normal reference standard is 15–65 pg/mL. HbA1c was determined by high-performance liquid chromatography (MQ-2000PT, China). Blood glucose and lipids were measured by Beckman Coulter AU 680 (Krefeld, Germany). Serum uric acid was measured by a DIMENSION LXR automatic analyzer (SIEMENS, Munich, Germany).

Assessment of DR

All participants were assessed for retinopathy by a fundus camera (AFC-330; NIDEX, Kyoto, Japan), slit lamp microscope (3020H; Keeler Ltd, Windsor, UK) and non-invasive optical coherence tomography (5000; Carl Zeiss, Dublin, CA, USA) within 2 h after blood samples were collected. According to the definitions derived from Wilkinson et al.[15], the patients were classified into three groups: non-diabetic retinopathy group, NPDR and proliferative diabetic retinopathy group. NPDR includes multiple manifestations: microaneurysm, hard exudates, cotton-wool spot and so on. Proliferative diabetic retinopathy is mainly the formation of neovascularization, which can lead to retinal detachment severely.

Statistical analysis

SPSS software version 24.0 (SPSS IBM Corporation, Armonk, NY, USA) was used to carry out statistical analyses. Normally distributed continuous variables are expressed as the

mean ± standard deviation, whereas non-normally distributed continuous variables are expressed as the interquartile range, and categorical variables are presented as the frequency. The characteristics of the participants between the non-diabetic retinopathy and NPDR groups were compared using the χ^2-test or Kruskal–Wallis test. PTH levels were classified into four groups based on quartiles in men (Q1: ≤23.74, Q2: 23.74–29.47, Q3: 29.47–37.30, Q4: >37.30) and in women (Q1: ≤24.47, Q2: 24.47–31.22, Q3: 31.22–39.49, Q4: >39.49), with the first quartile (Q1) representing the lowest quartile and the fourth quartile (Q4) being the highest. Multiple logistic regression analysis was carried out to determine the association between normal PTH and NPDR risk. PTH levels were equally divided into quartiles, with the first quartile (Q1) representing the lowest quartile and the fourth quartile (Q4) being the highest. In companion analyses, we categorized PTH in quartiles with the lowest quartile serving as the reference category. We analyzed the unadjusted model and the adjusted model for the variables. $P < 0.05$ was considered statistically significant (two-sided).

RESULTS

The clinical characteristics of the study participants are listed in Table 1. Among the 2,322 hospitalized type 2 diabetes mellitus patients, 54.0% were men and 46.0% were women. The mean serum PTH concentration was 29.87 ± 9.45 pg/mL in men and 30.76 ± 9.72 pg/mL in women. The NPDR group were significantly older, and had longer diabetes duration, lower free tri-iodothyronine and albumin, and higher HbA1c, SBP and serum creatinine than the non-diabetic retinopathy group ($P < 0.05$). PTH levels showed a stronger correlation with NPDR ($P < 0.001$).

The clinical characteristics of PTH quartile stratification in men and women with type 2 diabetes mellitus are shown in Table 2. Compared with the patients with the lowest PTH level, the patients with the highest PTH level were more likely to have higher HbA1c, BMI, SBP, DBP and magnesium, and lower phosphate ($P < 0.05$). The prevalence rate of NPDR showed a significantly decreasing trend both in men (37.9, 36.3, 34.0 *vs* 24.0%, $P < 0.05$) and women (43.2, 40.5, 31.1 *vs* 26.2%, $P < 0.05$) across the quartiles based on PTH. In addition, the prevalence of NPDR was significantly decreased in the last quartile of PTH compared with the first to third quartile in men (Figure 2a), and the prevalence of NPDR was significantly decreased in the last quartile of PTH compared with the first to second quartile in women (Figure 2b).

The results of logistic regression analysis are shown in Table 3. Compared with those in the first quartile, the odds ratios for NPDR in the last quartile were 0.516 (95% confidence interval [CI] 0.366–0.729) in men and 0.477 (95% CI 0.332–0.686) in women($P < 0.001$, unadjusted model). In the adjusted model, when PTH levels were stratified by quartile and clinical variables (i.e., age, duration of diabetes, BMI, blood pressure, HbA1c, smoking and drinking rate, low-density lipoprotein-

cholesterol, high-density lipoprotein-cholesterol, triglycerides, total cholesterol, sCr, thyroid-stimulating hormone, free tri-iodothyronine and free thyroid hormone) were adjusted, PTH remained an independent risk factor for NPDR. Compared with those in the first quartile, the prevalence of NPDR for the last quartile had odds ratios of 0.443 (95% CI 0.300–0.654) in men and 0.428 (95% CI 0.283–0.646) in women ($P < 0.001$, adjusted model). This suggested that the risk of NPDR in type 2 diabetes mellitus male patients with PTH <23.74 pg/mL is 2.26-fold higher than those with PTH >37.30 pg/mL, and the risk of NPDR in women with type 2 diabetes mellitus with PTH <24.47 pg/mL was 2.34-fold higher than that those with PTH >39.49 pg/mL.

DISCUSSION

In the present cross-sectional study, we recruited 2,322 type 2 diabetes mellitus patients to explore the relationship between NPDR and PTH levels within the normal reference range. The study found that the prevalence of NPDR showed a declining trend with the rise of PTH levels ($P < 0.05$). It also suggested that the patients with NPDR had a significantly lower PTH level than those without DR ($P < 0.001$). Multivariate logistic regression showed that the prevalence of NPDR for the last quartile had odds ratios of 0.488 (95% CI 0.335–0.712) in men and 0.442 (95% CI 0.296–0.661) in women ($P < 0.001$, adjusted model). This suggested that the risk of NPDR in male patients type 2 diabetes mellitus with PTH <23.74 pg/mL is 2.26-fold higher than those with PTH >37.30 pg/mL, and the risk of NPDR in women with type 2 diabetes mellitus with PTH <24.47 pg/mL was 2.34-fold higher than that those with PTH >39.49 pg/mL. This showed an independent, significant relationship between PTH levels and NPDR regardless of age, BMI, duration of type 2 diabetes mellitus, HbA1c and other variables included in the logistic regression.

So far, the association between normal parathyroid function and DR in type 2 diabetes mellitus patients has not been thoroughly studied. However previous clinical and preclinical studies have shown links between serum calcium, phosphate and DR; namely, high circulating calcium and phosphate levels are related to the increased risk of DR[14]. The evidence shows that the increase of serum calcium might trigger the self-regeneration cycle of ionized calcium influx and depolarization, resulting in increased retinal photoreceptor ellipsoid zone disruption in DR, leading to the impairment of retinal photoreceptor cell function and apoptosis[16,17]. Rupal *et al.*[14] and other scholars put forward that elevated phosphate has toxic effects on the vascular endothelium, which leads to retinopathy. *In vivo* and *in vitro* studies have found that endothelial cells exposed to high phosphate concentration reduce the content of endothelial nitric oxide synthase, resulting in decreased vasodilation and vascular dysfunction[18,19]. Other studies have reported that elevated phosphate induced endothelial cells to express and secrete interleukin-8, a cytokine in promoting vascular calcification[20]. The present study did not support the relationship between

Table 1 | Clinical characteristics of non-diabetic retinopathy and non-proliferative diabetic retinopathy in participants

Characteristics	Men with diabetes			Women with diabetes		
	NDR	NPDR	P	NDR	NPDR	P
n	839	414	–	691	378	–
Age (years)	57.6 ± 12.0	60.9 ± 10.4	<0.001*	61.8 ± 11.2	63.5 ± 10.0	0.024*
BMI (kg/m^2)	25.9 (23.7–28.1)	26.0 (23.8–28.2)	0.705	25.4 (23.4–27.9)	26.0 (23.4–28.0)	0.473
DM duration (years)	9.0 ± 6.9	12.2 ± 7.0	<0.001*	10.2 ± 7.2	13.7 ± 7.7	<0.001*
HbA1c (%)	8.1 (6.9–9.6)	8.5 (7.1–10.0)	0.007*	8.0 (6.8–9.4)	8.5 (7.3–10.0)	<0.001*
SBP (mmHg)	137.1 ± 17.6	140.8 ± 20.6	0.001*	138.8 ± 18.3	141.94 ± 20.7	0.005*
DBP (mmHg)	81.1 ± 11.7	80.9 ± 11.9	0.547	76.7 ± 10.6	75.8 ± 11.1	0.173
PTH (pg/mL)	31.99 ± 10.26	29.87 ± 9.45	<0.001*	33.92 ± 10.78	30.76 ± 9.72	<0.001*
Thyroid function						
TSH (mIU/L)	1.93 (1.34–2.85)	1.86 (1.25–2.83)	0.237	2.27 (1.38–3.61)	2.17 (1.44–3.29)	0.822
FT3 (mmol/L)	4.54 ± 0.96	4.39 ± 0.75	0.025*	4.18 ± 0.86	4.08 ± 0.96	0.029*
FT4 (mmol/L)	16.73 ± 2.91	16.30 ± 2.71	0.037*	16.05 ± 3.10	16.04 ± 2.81	0.829
Serum electrolyte (mmol/L)						
Sodium	140.57 ± 2.89	140.69 ± 2.85	0.380	141.2 ± 2.7	141.13 ± 2.87	0.914
Potassium	4.11 ± 0.39	4.16 ± 0.41	0.068	4.10 ± 0.35	4.15 ± 0.37	0.068
Magnesium	0.88 ± 0.08	0.88 ± 0.08	0.886	0.88 ± 0.08	0.88 ± 0.09	0.544
Calcium	2.26 ± 0.12	2.25 ± 0.13	0.426	2.27 ± 0.11	2.26 ± 0.13	0.086
Phosphate	1.18 ± 0.25	1.18 ± 0.21	0.227	1.23 ± 0.18	1.23 ± 0.21	0.628
Corrected calcium	2.30 ± 0.16	2.30 ± 0.17	0.190	2.28 ± 0.17	2.29 ± 0.18	0.194
Albumin (g/L)	40.46 ± 3.20	40.06 ± 2.92	0.112	40.33 ± 3.11	39.96 ± 2.97	0.024
Osteocalcin (μg/L)	8.73 ± 6.27	8.12 ± 6.21	0.060	11.82 ± 10.18	10.05 ± 7.59	<0.001*
25(OH)D$_3$ (ng/mL)	18.26 ± 7.30	18.83 ± 8.26	0.459	17.23 ± 6.82	17.72 ± 6.61	0.331
Lipid profile (mmol/L)						
LDL-c	2.61 ± 0.89	2.67 ± 1.00	0.448	2.84 ± 0.94	2.92 ± 1.05	0.385
HDL-c	1.14 ± 0.29	1.17 ± 0.33	0.268	1.30 ± 0.30	1.34 ± 0.35	0.136
FFA	0.43 ± 0.20	0.41 ± 0.25	0.007*	0.46 ± 0.23	0.45 ± 0.20	0.398
TC	4.43 ± 1.23	4.56 ± 1.32	0.144	4.78 ± 1.16	4.94 ± 1.34	0.138
TG	1.4 (1.0, 2.2)	1.3 (1.0, 2.3)	0.735	1.4 (1.0, 2.1)	1.5 (1.0, 2.2)	0.912
sUA (μmol/L)	335 (279, 393)	343 (285, 399)	0.198	293 (246, 341)	304 (240, 363)	0.059
sCr (μmol/L)	61.43 ± 18.48	69.95 ± 29.37	<0.001*	47.68 ± 15.76	55.35 ± 23.30	<0.001*
Smoking history	441 (52.6%)	237 (57.2%)	0.108	8 (1.2%)	0 (0%)	0.036*
Drinking history	422 (50.3%)	199 (48.1%)	0.446	11 (1.6%)	5 (1.3%)	0.729

Kruskal–Wallis *H*-test or χ^2-test. Normally distributed variables are expressed as the mean ± standard deviation, non-normal variables are expressed as the median (interquartile range) and categorical variables are expressed as the percentage (%). 25(OH)D$_3$, 25-hydroxyvitamin D$_3$; BMI, body mass index; BP, blood pressure; DM, diabetes mellitus; FFA, free fatty acid; FT3, free triiodothyronine; FT4, free thyroxine; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PTH, parathyroid hormone; sCr, serum creatinine; sUA, serum uric acid; TC, total cholesterol; TG, triglyceride; TSH, thyroid-stimulating hormone. *Statistically significant.

serum calcium, phosphate, 25-hydroxyvitamin D$_3$ and NPDR, the reasons for inconsistency with the above research might be as follows. First, we did not include patients with all types of DR, such as patients with severe proliferative retinopathy. Second, the prevalence of DR in the hospitalized patients we studied was higher than that in the general population. In addition, the reference ranges of these indicators measured by different laboratories are not identical.

Increasing clinical and preclinical studies have researched the link between PTH and diabetes. In recent years, retrospective and prospective studies have shown that PTH is associated with insulin resistance[21], insulin sensitivity[22–24], β-cell dysfunction[22–24] and abnormal blood glucose during pregnancy[25]. In the cell experiment, PTH treatment induced insulin-stimulated glucose uptake and decreased protein kinase B activity (phosphorylated protein kinase B/total protein kinase B protein expression). In addition, PTH promotes the phosphorylation of insulin receptor substrate-1 on serine 307, thus inhibiting insulin signal transduction[26]. A study carried out in Eastern China shows that the level of intact parathyroid hormone (iPTH) in patients with diabetic nephropathy is lower than that in patients with non-diabetic nephropathy[27]. Contrary to the findings of the previous study, a study found that PTH levels were significantly increased in patients with diabetic chronic kidney disease at the Antalya Research and Training Hospital ($P < 0.001$)[28]. Furthermore, there was a positive correlation between daily proteinuria

Table 2 | Clinical characteristics of parathyroid hormone quartile stratification in men and women with type 2 diabetes mellitus

Characteristics	Men with diabetes					Women with diabetes				
	Q1	Q2	Q3	Q4	P	Q1	Q2	Q3	Q4	P
n	314	314	312	313	-	268	268	267	266	-
Age (years)	59 ± 12	59 ± 11	59 ± 11	57 ± 12	0.111	62 ± 11	61 ± 11	64 ± 10	62 ± 12	0.009*
BMI (kg/m^2)	25.4 (23.6–27.8)	26.0 (23.7–28.0)	26.0 (23.8–28.1)	26.4 (24.2–28.7)	0.005*	25.5 (23.2–28.0)	25.4 (23.1–27.6)	25.2 (23.3–27.6)	26.1 (23.8–28.9)	0.009*
DM duration (years)	10.2 ± 6.9	9.9 ± 6.9	10.4 ± 7.3	9.8 ± 7.3	0.611	12.0 ± 7.4	11.2 ± 7.2	11.5 ± 7.5	11.0 ± 8.0	0.205
HbA1c (%)	8.7 (7.1–10.0)	8.3 (7.3–9.9)	8.0 (6.9–9.6)	7.9 (6.8–9.3)	0.008*	8.8 (7.2–10.2)	8.3 (7.1–9.8)	7.8 (6.8–9.3)	7.8 (6.8–9.3)	0.001*
SBP (mmHg)	136 ± 17	137 ± 19	140 ± 20	140 ± 18	0.008*	138 ± 18	138 ± 21	141 ± 18	143 ± 19	0.002*
DBP (mmHg)	79 ± 11	81 ± 12	81 ± 12	83 ± 12	0.011*	75 ± 10	76 ± 12	76 ± 10	78 ± 10	0.003*
Thyroid function										
TSH (mIU/L)	1.8 (1.2–2.7)	1.9 (1.3–2.8)	2.0 (1.3–2.9)	1.9 (1.4–2.9)	0.155	2.0 (1.0–2.3)	2.2 (1.4–3.6)	2.3 (1.3–3.4)	2.4 (1.5–3.6)	0.119
FT3 (mmol/L)	4.5 ± 1.3	4.5 ± 0.8	4.5 ± 0.7	4.5 ± 0.7	0.223	4.2 ± 1.1	4.1 ± 0.7	4.1 ± 0.9	4.2 ± 0.7	0.323
FT4 (mmol/L)	16.8 ± 3.4	16.6 ± 3.0	16.5 ± 2.4	16.4 ± 2.6	0.715	16.3 ± 3.4	16.0 ± 2.6	16.0 ± 3.4	16.0 ± 2.4	0.521
Serum electrolyte (mmol/L)										
Sodium	140 ± 3	141 ± 2	141 ± 3	141 ± 3	0.047*	141 ± 3	141 ± 3	141 ± 3	141 ± 2	0.034*
Potassium	4.2 ± 0.4	4.1 ± 0.4	4.1 ± 0.4	4.1 ± 0.4	0.161	4.1 ± 0.3	4.1 ± 0.3	4.1 ± 0.4	4.1 ± 0.4	0.338
Magnesium	0.86 ± 0.08	0.89 ± 0.08	0.89 ± 0.09	0.89 ± 0.07	<0.001*	0.87 ± 0.09	0.87 ± 0.08	0.88 ± 0.08	0.89 ± 0.08	0.008*
Calcium	2.26 ± 0.13	2.27 ± 0.12	2.26 ± 0.13	2.25 ± 0.13	0.330	2.27 ± 0.13	2.28 ± 0.11	2.26 ± 0.11	2.28 ± 0.13	0.418
Phosphate	1.22 ± 0.34	1.18 ± 0.17	1.19 ± 0.20	1.14 ± 0.19	<0.001*	1.25 ± 0.20	1.24 ± 0.22	1.23 ± 0.17	1.20 ± 0.17	0.007*
Lipid profile (mmol/L)										
LDL-c	2.6 ± 0.9	2.7 ± 1.0	2.6 ± 0.9	2.6 ± 0.9	0.602	2.9 ± 1.0	2.9 ± 0.9	2.8 ± 0.9	2.9 ± 1.1	0.416
HDL-c	1.1 ± 0.3	1.2 ± 0.3	1.1 ± 0.3	1.2 ± 0.3	0.130	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	0.938
FFA	0.40 ± 0.19	0.41 ± 0.19	0.44 ± 0.19	0.46 ± 0.21	0.004*	0.45 ± 0.21	0.46 ± 0.21	0.45 ± 0.20	0.48 ± 0.20	0.134
TC	4.4 ± 1.2	4.5 ± 1.2	4.5 ± 1.3	4.5 ± 1.2	0.736	4.9 ± 1.3	4.9 ± 1.2	4.7 ± 1.1	4.9 ± 1.3	0.409
TG	1.3 (1.0–2.2)	1.4 (0.9–2.3)	1.4 (1.0–2.2)	1.5 (1.0–2.2)	0.794	1.5 (1.0–2.3)	1.4 (1.0–2.1)	1.3 (1.0–2.0)	1.5 (1.1–2.3)	0.121
sUA (μmol/L)	331 (276–393)	331 (280–385)	340 (277–402)	345 (298–397)	0.348	291 (237–351)	279 (236–334)	303 (247–356)	304 (257–365)	0.009*
sCr (μmol/L)	64.2 ± 28.5	61.4 ± 14.9	64.7 ± 20.6	66.7 ± 25.7	0.007*	50.9 ± 20.6	48.2 ± 12.5	49.9 ± 17.4	52.8 ± 24.4	0.762
Smoking history	172 (54.8%)	167 (53.2%)	167 (53.5%)	171 (54.6%)	0.964	2 (0.7%)	0 (0%)	4 (1.5%)	2 (0.8%)	0.257
Drinking history	152 (48.4%)	144 (45.9%)	154 (49.4%)	170 (54.3%)	0.144	5 (1.9%)	4 (1.5%)	4 (1.5%)	3 (1.1%)	0.920
NPDR	119 (37.9%)	114 (36.3%)	106 (34%)	75 (24%)	0.001*	116 (43.2%)	109 (40.5%)	83 (31.1%)	70 (26.2%)	<0.001*

Kruskal–Wallis *H*-test or χ^2-test. Normally distributed variables are expressed as the mean ± standard deviation, non-normal variables are expressed as the median (interquartile range) and categorical variables are expressed as the percentage (%). BMI, body mass index; BP, blood pressure; DM, diabetes mellitus; FFA, free fatty acid; FT3, free triiodothyronine; FT4, free thyroxine; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PTH, parathyroid hormone; Q1, quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4; sCr, serum creatinine; sUA, serum uric acid; TC, total cholesterol; TG, triglyceride; TSH, thyroid-stimulating hormone. *Statistically significant.
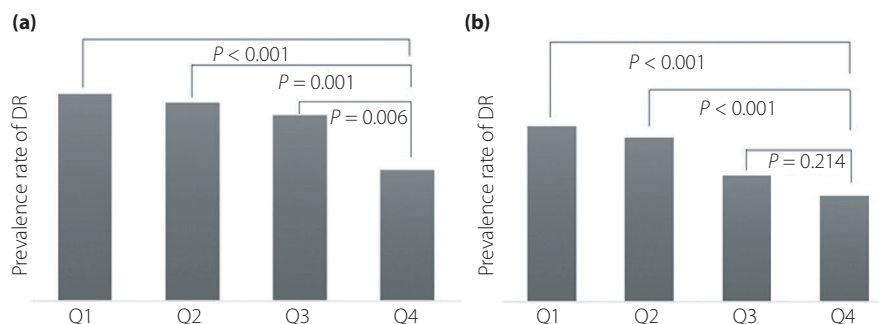
Figure 2 | Prevalence rate of diabetic retinopathy (DR) in parathyroid hormone quartiles (Q) in (a) men with type 2 diabetes and (b) women with type 2 diabetes.

Table 3 | Unadjusted and adjusted odds ratios of the quartiles of parathyroid hormone levels for non-proliferative diabetic retinopathy in participants

Quartiles	Unadjusted model		Adjusted model	
	OR (95% CI)	P	OR (95% CI)	P
Men				
Q1 (≤23.74)	–	–	–	–
Q2 (23.74–29.47)	0.934 (0.676–1.291)	0.680	0.912 (0.636–1.309)	0.619
Q3 (29.47–37.30)	0.843 (0.608–1.169)	0.307	0.754 (0.522– 1.088)	0.131
Q4 (>37.30)	0.516 (0.366–0.729)	<0.001*	0.443 (0.300–0.654)	<0.001*
Women				
Q1 (≤24.47)	–	–	–	–
Q2 (24.47–31.22)	0.871 (0.618–1.228)	0.430	0.961 (0.657–1.406)	0.837
Q3 (31.22–39.49)	0.601 (0.422–0.857)	0.005	0.680 (0.458–1.010)	0.056
Q4 (>39.49)	0.477 (0.332–0.686)	<0.001*	0.428 (0.283–0.646)	<0.001*

Logistic regression analysis. Adjusted for age, body mass index, duration of type 2 diabetes mellitus, glycated hemoglobin, blood pressure, smoking and drinking rate, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglyceride, total cholesterol, serum creatinine, thyroid-stimulating hormone, free triiodothyronine and free thyroxine. CI, confidence interval; OR, odds ratio; Q1, quartile 1; Q2, quartile 2; Q3, quartile 3; Q4, quartile 4. *Statistically significant.

and PTH levels in patients with diabetic nephropathy[29]. Compared with the normal control group, postmenopausal women with primary hyperparathyroidism have retinal microvascular stenosis[30], which might lead to retinal ischemia and further retinopathy. On the contrary, a study by Hekimsoy et al.[31] suggested that low parathyroid hormone concentrations might be associated with reduced retinal perfusion in patients with hypoparathyroidism. However, so far, the relationship between normal parathyroid hormone levels and DR in patients with type 2 diabetes mellitus has not been thoroughly studied. The key finding of the present study was that the normal level of PTH in patients with type 2 diabetes mellitus is negatively correlated with the risk of NPDR, and the following possible basic mechanisms are summarized.

- High concentration of glucose and insulin deficiency inhibits the secretion of PTH. In an *in vitro* study involving bovine parathyroid cells, cells were cultured in medium with different concentrations of glucose and insulin, and the concentration of PTH was measured after a period of time. It was

found that the secretion of parathyroid hormone was inhibited in a dose-dependent manner, with the increase of glucose concentration in culture medium. After being cultured in insulin-free medium for 96 h, the protein content of bovine parathyroid cells decreased significantly by 12–15%[32]. Other studies have also confirmed that insulin is required to maintain the secretion of parathyroid hormone[33]. In a study of 98 individuals with type 2 diabetes mellitus and end-stage renal disease, HbA1c levels were significantly negatively correlated with serum iPTH levels. Poor blood glucose control (HbA1c >7.0%) is related to the decrease of serum iPTH level, and good blood glucose control (HbA1c <7.0%) is related to the increase of serum iPTH[34,35].

- Improved endothelial function. Studies have shown that intermittent parathyroid hormone can improve endothelium-dependent relaxation in elderly rodents[36]. Vascular dysfunction is largely due to reduced bioavailability or production of endothelium-derived vasodilator nitric oxide. The decrease in age-related nitric oxide was attributed to the low expression

or activity of endothelial nitric oxide synthase[37]. In this analysis, daily administration of PTH1-34 (a PTH analog) in elderly rodents can increase the expression and activity of endothelial nitric oxide synthase in arteries, and even return to a level similar to that of young arteries[36]. We know that endothelial dysfunction plays an important role in the development of DR. Therefore, the level of PTH might be closely related to DR through endothelial function.

The present study had several limitations that need to be explained. First of all, this retrospective study cannot infer causality. Second, all recruited patients were hospitalized in one area, so the results do not represent populations in other areas. Third, there is not enough experimental evidence to explain the relationship between them. In summary, the present study shows that normal low levels of PTH are significantly associated with NPDR risk. This might mean that lower PTH levels in type 2 diabetes mellitus patients are a potential clinical indicator to identify DR. The relationship between PTH and DR will open up a new research field, and the treatment of PTH still needs careful and comprehensive consideration. Large-scale prospective cohort studies are still required to determine the causal relationship between PTH and DR.

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DISCLOSURE

The authors declare no conflict of interest.

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