Published in final edited form as:

Am J Med Sci. 2017 October ; 354(4): 355–361. doi:10.1016/j.amjms.2017.07.003.

Association of bone metabolic markers with diabetic retinopathy and diabetic macular edema in elderly Chinese individuals with type 2 diabetes mellitus

Xiao Zhang, MD^{1,†}, Jingyun Yang, PhD^{†,2,3,4,5}, Yong Zhong, MD^{†,1}, Lingling Xu, MD^{6,†}, Ou Wang, MD^{6,†}, Ping Huang, MD^{7,†}, Chunying Li, MD^{8,†}, Bin Qu, MD^{7,†}, Jing Wang, MD⁷, Caixia Zheng, MD⁷, Meng Niu, MD⁸, and Weihong Yu, MD^{1,*}

¹Department of Ophthalmology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China

²Division of Statistics, School of Economics, Shanghai University, Shanghai, China

³Research Center of Financial Information, Shanghai University, Shanghai, China

⁴Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, IL, USA

⁵Department of Neurological Sciences, Rush University Medical Center, Chicago, IL, USA

⁶Department of Endocrinology, Key Laboratory of Endocrinology of the Ministry of Health, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China

⁷Department of Ophthalmology, Traditional Chinese Medicine Hospital of Muping District of Yantai City, Yantai, Shandong, China

⁸Department of Endocrinology, Traditional Chinese Medicine Hospital of Muping District of Yantai City, Yantai, Shandong, China

Abstract

Background—Diabetic retinopathy (DR) is a common and specific microvascular complication of diabetes. The association of bone metabolic markers with the risk of DR and diabetic macular edema (DME) is unclear.

Materials and Methods—We investigated the association between bone turnover markers commonly examined in a clinical setting and DR and DME risk in elderly Chinese patients with type 2 diabetes mellitus (T2DM). A total of 408 patients aged 55 to 70 years old with T2DM were included. We first performed univariable logistic regression followed by multivariable logistic regression that included variables selected using purposeful selection.

Corresponding to: Weihong Yu, Department of Ophthalmology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, No.1 Shuaifuyuan Road, Dongcheng District, Beijing 100730, China, Tel: 86-10-69156351, Fax: 86-10-69156351, yuweihongye@foxmail.com.

[†]These authors contributed equally to this paper

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Competing interests: The authors declare no competing financial interests.

Results—Fasting blood glucose ($P=0.007$) and duration of diabetes ($P<0.0001$) were significantly associated with DME in multivariable logistic regression; however, the association of β -CTx with DME risk was not statistically significant ($P=0.053$). Sex-stratified analysis showed that β -CTx was significantly associated with DME only in female subjects ($P=0.011$).

Conclusions— β -CTx had no significant association with DR. It was significantly associated with DME in female T2DM patients but not in male T2DM patients. More prospective studies with larger sample sizes are warranted to validate our findings.

Keywords

diabetic retinopathy; diabetic macular edema; bone metabolic markers; β -CTx

Introduction

Diabetic retinopathy (DR) is a frequent and specific microvascular complication of diabetes. It continues to be the leading cause of blindness in many countries [1]. Vision loss generally develops as a sequela of diabetic macular edema (DME) and neovascularization of the retina, leading to vitreous hemorrhage and retinal detachment. In many countries, DR is a common cause of vision loss in elderly populations and is the most frequent cause of preventable blindness among individuals of working age [2]. It was estimated that in the United States, 28.5% of diabetes patients had DR and 2.7% had clinically significant DME [3]. DR considerably compromises patients' ability to perform routine activities, thereby reducing their quality of life [4].

The bone is constantly being remodeled to maintain a healthy skeleton structure as per an individual's needs. Bone remodeling products in the body that can be detected using blood and urine tests are useful as markers of bone metabolism. Previous research has revealed an important association between bone metabolism and energy metabolism [5], which influences the risk of DR and DME. For instance, low osteocalcin concentrations were reported to be associated with obesity as well as with type 2 diabetes mellitus (T2DM) [6]. Moreover, diabetes mellitus (DM) patients are more likely to experience bone fragility problems leading to issues such as higher fracture risk and delayed fracture healing [7], indicating a possible link between bone metabolism with DM and its associated complications such as DR and DME. In addition, a previous study of 3,654 older Australians found that DR was associated with all fractures combined and with proximal humerus fracture [8]. The interrelationship between DM and bone metabolism and between bone metabolism and DR suggests that bone metabolic markers can be used to assess DM risk and subsequent complications such as DR and DME. Therefore, in this study, we sought to determine whether bone metabolism, as indicated by bone turnover markers such as serum procollagen type 1 nitrogenous propeptides (P1NP), β -CTx, 25-hydroxyvitamin D (25(OH)D) and fibroblast growth factor 21 (FGF21), are associated with the risk of DR or DME in elderly Chinese patients with T2DM.

Methods

Study Participants

This cross-sectional study enrolled T2DM patients who visited the clinic of the Department of Ophthalmology of the Traditional Chinese Medicine Hospital in the Muping District of Yantai City in Shandong Province, China, between October 2012 and May 2013. For each patient, a personal interview was conducted to collect basic demographic data regarding age, sex, height, body weight, education level as well as smoking and drinking history. Information about past medical history, duration of diabetes (years), and the diabetes treatment was also collected. The study population comprised 408 consecutive T2DM patients between ages of 55 and 70 years. Patients were excluded from this study if they were < 55 years old. Those who had a history of eye diseases known to affect vision, such as optic disk atrophy; signs of significant media opacification as determined by slit-lamp examination through a dilated pupil; or contraindication to pupil dilation were also excluded. Informed consent was obtained from each patient for participation in the study. All experiments were performed as per the relevant guidelines and regulations of the Peking Union Medical College Hospital. The study procedures were carried out in accordance with the approved guidelines, and the study was approved by the ethics committee of the Peking Union Medical College Hospital.

Diagnosis of DR and ME

The 7-field Early Treatment Diabetic Retinopathy Study (ETDRS) color fundus photographs were taken by a trained ophthalmic technician using VISUCAM Lite Digital Fundus Camera (Carl Zeiss Meditec, AG, Jena, Germany). All the fundus photographs were taken after pupil dilation. The images were read by 2 independent readers using the retinopathy classification and the macular edema classification based on the UK National Health Service (NHS) Diabetic Eye Screening Program [7], and a senior reader arbitrated the discrepant grades. According to the screening scale, R0 represents no DR, R1 and R1.5 represent background DR, R2 and R2.5 represent pre-proliferative DR, and R3 represents proliferative DR (See Supplementary Table 1). According to the ETDRS classification protocol, DME was classified as clinically significant macular edema (CSME) if there was retinal thickening at or within 500 μ m of the center of the macula or if there were hard exudates at or within 500 μ m of the center of the macula associated with thickening of the adjacent retina or zones of retinal thickening (1 disc area in size), at least part of which was within 1 disc diameter of the center [9]. Retina thickening was confirmed using optical coherence topography (OCT, Cirrus HD-OCT 400, Carl Zeiss Meditec, Dublin, CA, USA).

Other Measurements

Patients fasted overnight (for 8–12 hours) before their blood samples and urine specimens were collected the next morning. Blood samples were drawn from the cubital vein and prepared for immediate analysis or for storage at -80°C for further analysis. Fasting blood glucose and postprandial blood glucose, plasma total cholesterol (TC) and triglyceride (TG), creatinine, and glycosylated hemoglobin (HbA1C) levels were measured per the standard protocols. Serum concentrations of β -CTx, P1NP and 25(OH)D were determined using a fully automated Roche electrochemiluminescence system (E170, Roche Diagnostics, Basel,

Switzerland) with a single-step sandwich electrochemiluminescence immunoassay (ECLIA). All tests were performed using standard automated methods. The detection limits of β -CTx, P1NP and 25(OH)D were 0.01 ng/mL, 5 ng/mL and 4 ng/mL, respectively. Microalbuminuria (MAU) was measured using fresh morning spot urine with an immunoturbidimetric assay. Intra- and inter-assay coefficients of variation (CV) for detection of the serum levels were as follows: CV=1.2~4.9% and 4.3~6.5% in the measurement of P1NP; CV=1.6~3.0% and 1.3~4.3% in the measurement of β -CTx; CV=1.7~7.8% and 2.2~10.7% in the measurement of 25(OH)D, respectively. FGF21 and osteonectin were measured using the sandwich ELISA kits (Cloud-Clone Corp, Houston, TX, USA). The intra- and inter-assay CV of the FGF21 were 2.9% and 3.7%, respectively, while the intra- and inter-assay CV of the osteonectin were 3.4% and 10.5%, respectively. Blood pressure, waist circumference, hip circumference, and body weight were also measured according to standard procedures. The β -CTx were severely skewed; therefore, we log-transformed the variable in the analyses, where appropriate.

Statistical analysis

For each participant, we used the DR and DME data from the more severely affected eye. We first compared the demographic and clinical characteristics of the subjects with and without DME and DR. Chi-square test or Fisher's exact test, as appropriate, was used to compare categorical variables, while Student's t-test or Wilcoxon rank-sum test, as appropriate, was used for comparing continuous variables.

The association of bone metabolic markers and other factors with the risk of DR and DME was modeled using logistic regression. We first performed univariable logistic regression followed by purposeful selection in logistic regression to select important variables for risk-factor modeling of DR and DME. The objective of purposeful selection is similar to that of several existing methods for variable selection in logistic regression, such as the forward selection, backward selection, and stepwise selection. However, purposeful selection follows a slightly different logic and can select not only significant variables but also confounders. Simulation studies have indicated that purposeful selection has found be to superior to the existing methods [10]. In purposeful selection, the presence of DR or DME is the response variable, and we included all available variables, as presented in Table 1, as the candidate variables for variable selection except blood glucose medication, the estimation of which was unreliable, based on the univariate logistic regression analysis. We used all the recommended settings for the inclusion and retention of variables, confounding criteria and inclusion of non-candidate variables [10]. We have presented the results of the multivariable logistic regression analysis using purposeful selection.

All data analyses were performed using R (www.R-project.org) or SAS version 9.3 (SAS Institute Inc., Cary, NC, USA) software, and we have considered P values <0.05 to be statistically significant.

Results

A total of 408 patients were included in the analyses. Their mean age was 61.3 $\pm$ 4.1 (range 55 - 69 years), and 35.3% were men. The mean time since the diagnosis of T2DM was

10.1±6.2 years (range 1–28 years). A total of 229 patients (56.1%) had DR, and of them, 59 (25.8%) had DME. The comparison of the patients with and without DR and DME is presented in Table 1. The DR patients had higher average values for HbA1C (8.3±1.7 vs. 7.7±1.7; $P=0.0005$), MAU (41.0±67.9 vs. 19.3±28.2; $P<0.0001$), and fasting plasma glucose (9.7±3.3 vs. 8.7±2.7; $P=0.0004$); had a longer average duration of diabetes (12.3±6.1 vs. 7.1±4.9 years; $P<0.0001$); and had lower β -CTx levels (0.35±0.2 vs. 0.40±0.2; $P=0.002$). Similarly, the DME patients had higher average values for HbA1C (9.0±1.9 vs. 7.8±1.7; $P<0.0001$), MAU (68.8±83.4 vs. 25.1±46.5; $P=0.0003$), and fasting plasma glucose (11.4±3.7 vs. 8.9±2.8; $P<0.0001$); had a longer duration of diabetes (15.6±5.2 vs. 9.0±5.7 years; $P<0.0001$) and had lower β -CTx levels (0.3±0.1 vs. 0.4±0.2; $P=0.0008$). In addition, we also found that patients with DME had lower P1NP levels (36.9±14.1 vs. 42.0±21.3; $P=0.026$; Table 1). We did not find significant differences in the distribution of the other variables between the subjects with and without DME, and between those with and without DR. Comparing the 59 patients who had DME and the 170 patients who had DR but not DME, we found that on average, patients with DME had higher MAU (68.8±83.4 vs. 31.4±60.0; $P=0.003$) and FBG (11.4±3.7 vs. 9.1±3.0; $P<0.0001$) levels; however, there was no significant difference in the AZGP1 levels ($P=0.22$).

Univariable logistic regression showed that MAU, β -CTx, duration of diabetes, HbA1C and fasting blood glucose were associated with the risk of both DR and DME (all $P=0.003$; Table 2). In addition, we found that systolic blood pressure (SBP) was significantly associated with DR but not with DME ($P=0.035$), while cholesterol was significantly associated with DME ($P=0.003$) but not with DR.

Multivariable logistic regression using purposeful selection indicated that the duration of diabetes (odds' ratio [OR]=1.16, 95% confidence interval [CI]: 1.11–1.22; $P<0.0001$) was significantly associated with the risk of DR. On the other hand, fasting blood glucose (OR=1.2, 95% CI: 1.0–1.4; $P=0.011$) and duration of diabetes (OR=1.2, 95% CI: 1.2–1.3; $P<0.0001$) were associated with an increased risk of DME. In addition, we found that men had a lower risk of DME than women (OR=0.3, 95% CI: 0.1–0.7; $P=0.006$; Table 3). The association of β -CTx (log-scaled) with DME was no longer statistically significant (OR=0.4, 95% CI: 0.1–1.0; $P=0.053$). Since we observed differences in the DME risk of the male and female subjects, we performed additional sex-stratified analysis, controlling for all the factors, except sex, that were retained by purposeful selection. In both male and female subjects, that the duration of diabetes was significantly associated with the risk of DME. We observed that β -CTx (log-scaled) showed a significant association with DME only in female subjects (OR=0.1, 95% CI: 0.0–0.6; $P=0.011$; online Supplementary Table 1). Distribution of β -CTx by sex is presented in Figure 1, which indicated that the average β -CTx level was higher in female T2DM patients than in male T2DM patients (0.4±0.2 vs. 0.3±0.1; $P<0.0001$).

Discussion

In this paper, we investigated whether the bone metabolic markers commonly examined in clinical settings were associated with DR and DME risk in elderly Chinese individuals with T2DM. Although β -CTx showed a significant association with DR in univariable analysis, it

was not selected during purposeful selection in multivariable logistic regression analysis of DR. In the sex-stratified analysis of DME, we found that β -CTx was significantly associated with DME in female T2DM patients but not in male T2DM patients. To the best of our knowledge, this is the first study reporting a sex-different effect of β -CTx on the risk of DME.

DM can affect bone metabolism [11]. Previous studies have reported that metabolic and endocrine changes caused by diabetes can negatively affect bone quantity and density [12]. It was also found that T2DM and MetS are associated with altered a bone turnover rate. In particular, a decreased bone turnover was reported in some T2DM cohort studies and in Leptin receptor-deficient db/db mice, a T2DM animal model [5, 13]. On the other hand, accumulating evidence has also demonstrated that the bone is an endocrine regulator of energy metabolism. For example, osteocalcin hormone secreted by the osteoblasts is a key regulator of glucose and fat metabolism [14, 15]. Despite the interrelationship between bone metabolism and the risk of diabetes, few studies have been conducted to identify the biomarkers of bone metabolism that may be associated with DR or DME.

β -CTx is the beta form of C-terminal telopeptide of type I collagen and is released into the circulation during bone resorption. Therefore, serum β -CTx is a highly sensitive and specific marker for bone resorption [16, 17]. In this study, univariate logistic regression analysis indicated a significant association between β -CTx and DME. However, this association disappeared in multivariate logistic regression, implying that the association might have been due to the confounding effect of the other variables. In fact, we observed a significant negative correlation between β -CTx and the duration of diabetes ($\rho = -0.2$; $P < 0.0001$). The mechanisms underlying the sex-specific association are still unclear. We found that the average β -CTx level was higher in female T2DM patients than in male T2DM patients (Figure 1), indicating that diabetes may have a sex-specific effect on bone metabolism. Medications commonly used for the treatment of T2DM may also have a sex-differential impact on bone metabolism, especially in postmenopausal women [18]. Menopause conditions and sex-hormone levels might also play a role. Inflammation might also be involved as it has a significant effect on bone [19]. Elevated levels of inflammatory mediators, particularly TNF, are among the striking features of diabetes [20], and may have sex-specific effect on bone metabolism. All these factors together might partly explain the sex-specific association between β -CTx and DME risk. Few studies have been conducted on the role of bone metabolism in influencing DME risk; moreover, due to the cross-sectional study design, we could not assess whether bone turnover, as determined using β -CTx levels, is a cause or a consequence of DME. Further animal studies are greatly warranted to explore the mechanisms underlying the association of β -CTx and DME risk.

Among the several biochemical markers of bone turnover, β -CTx and P1NP are markers of the resorption and formation of collagen, and are believed to reflect the early changes during bone turnover [21]. P1NP is a circulating protein released by the osteoblasts during the synthesis of collagen type I and is predominantly located in the bone matrix [22]. Previous studies have shown that P1NP levels are lower in postmenopausal women with T2DM [23], and that P1NP levels are significantly and negatively correlated with the initial HbA1C levels and the duration of diabetes in postmenopausal women [21]. In our study, we found

that on average, DME patients had lower PINP levels; there was no significant difference in the mean PINP levels between subjects with and without DR. However, PINP was selected as an important confounder by purposeful selection for both DR and DME risk.

Our study has limitations. The sample size is limited, especially for the sex-stratified analysis. More studies with larger sample sizes are needed to validate our findings. We used cross-sectional data, and therefore could not analyze the long-term effects of β -CTX on the risk of DME. The patients included in this study were from a single hospital, and it remains to be determined whether our findings can be generalized to patients from other institutions. We only included various bone turnover markers commonly examined in a clinical setting; more bone turnover markers need to be studied for their relationship with DR and DME risk and their application in a clinical setting. History or medication of osteoporosis and bone density would also have been helpful in assessing the relationship between bone turnover markers and DR and DME risk. Unfortunately, we did not collect these data in this study. However, we are currently collecting this information as part of a follow-up study that is underway wherein we will assess the roles of these variables with regard to the DR and DME risk. It would also be interesting to investigate the relationship between the examined markers and future outcomes, such as the association of fractures and DR and DME progression. We are conducting a follow-up study involving the patients included in this study. Data are being collected, and we will report the study findings in a separate manuscript.

Conclusions

In summary, we analyzed the association of bone metabolic markers with the risk of DR and DME. We found that β -CTX had no significant association with DR, but was significantly associated with DME in female T2DM patients. More prospective studies with larger sample sizes are warranted to validate our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Source of Funding: None

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Figure 1. Distribution of β -CTx by sex in subjects with and without DME

P-values were obtained using multiple logistic regression controlling for fasting blood glucose, HbA1C, MAU, duration of diabetes, PINP, AZGP1, and FGF21

AZGP1, zinc-alpha-2-glycoprotein; β -CTx, beta C-terminal telopeptide of collagen type I; DME, diabetic macular edema; FGF21, fibroblast growth factor 21; HbA1C, hemoglobin A1C; MAU, microalbuminuria, PINP, procollagen type 1 N-terminal propeptide.

Table 1

Basic characteristics of the study participants.

	DR (n=229)	No DR (n=179)	P	DME (n=59)	No DME* (n=343)	P
Age	61.4±4.0	61.2±4.1	0.622	61.1±4.5	61.3±4.0	0.630
Sex, male	82 (35.8%)	62 (34.6%)	0.806	14 (23.7%)	127 (37.0)	0.048
Waist circumference, cm	97.8±9.5	98.3±8.2	0.614	98.2±9.1	97.9±9.0	0.866
Hip circumference, cm	101.0±6.5	102.0±7.0	0.117	102.8±6.3	101.1±6.7	0.071
BMI, kg/m ²	27.3±3.8	27.6±5.5	0.513	28.1±4.0	27.3±4.7	0.265
Smoking history	27 (11.9%)	26 (14.5%)	0.435	4 (6.8%)	46 (13.5%)	0.150
Drinking history	18 (8.0%)	20 (11.2%)	0.263	4 (6.9%)	33 (9.7%)	0.496
SBP, mmHg	138.6±16.8	135.3±13.6	0.029	138.0±18.4	137.0±15.0	0.691
DBP, mmHg	83.9±8.5	83.4±8.7	0.587	83.7±9.4	83.7±8.5	0.970
Hypertension, yes	120 (52.9%)	89 (49.7%)	0.529	31 (52.5%)	175 (51.3%)	0.862
HbA1C, %	8.3±1.7	7.7±1.7	0.0005	9.0±1.9	7.8±1.7	<0.0001
Duration of diabetes, years	12.3±6.1	7.1±4.9	<0.0001	15.6±5.2	9.0±5.7	<0.0001
Triglyceride, mmol/L	1.5±2.4	1.4±0.9	0.524	1.3±0.5	1.4±2.1	0.183
Creatinine, μ mol/L	74.1±14.8	72.7±12.9	0.302	74.5±16.1	73.2±13.3	0.561
eGFR [#]	87.7±18.7	93.8±57.7	0.175	87.5±20.2	90.7±43.6	0.362
MAU, mg/24h	41.0±67.9	19.3±28.2	<0.0001	68.8±83.4	25.1±46.5	0.0003
25(OH)D, nmol/L	45.5±18.3	46.4±18.1	0.630	43.9±17.5	46.2±18.4	0.392
PINP, ng/mL	40.5±19.9	41.9±21.3	0.507	36.9±14.1	42.0±21.3	0.026
AZGP1, ng/mL [#]	1412.0±463.6	1413.7±150.1	0.971	1357.1±340.5	1422.8±474.8	0.223
FGF21, ng/mL	28.1±12.1	28.2±6.9	0.935	30.1±20.6	27.9±7.2	0.441
Osteonectin, ng/mL	890.8±363.2	947.1±377.7	0.133	922.9±337.6	910.6±374.2	0.820
β -CTX [*] , ng/mL	0.4±0.2	0.40±0.2	0.002	0.3±0.1	0.4±0.2	0.0008
Fasting blood glucose, mmol/L	9.7±3.3	8.7±2.7	0.0004	11.4±3.7	8.9±2.8	<0.0001
Blood glucose medication, yes	228 (99.6%)	164 (92.7%)	0.0001	59 (100%)	327 (95.9%)	0.103
Cholesterol, mmol/L	5.7±1.5	5.7±1.2	0.645	6.2±1.5	5.6±1.3	0.002

Results were presented as mean±SD for continuous variables and n (%) for categorical variables.

* Six participants had missing data regarding DME.

AZGP1, zinc-alpha-2-glycoprotein; β-CTX, beta C-terminal telopeptide of collagen type I; CKD-EPI, Cockcroft-Gault equation; DBP, diastolic blood pressure; DME, diabetic macular edema; DR, diabetic retinopathy; FGF21, Fibroblast growth factor 21; HbA1C, hemoglobin A1C; MAU, microalbuminuria; PINP, procollagen type 1 N-terminal propeptide; SBP, systolic blood pressure.

eGFR was estimated using CKD-EPI.

One patient with a high level of AZGP1 was excluded from analysis.

* β-CTX was log-transformed in the test to account for skewness in the data.

Table 2

Association with presence of diabetic retinopathy and macular edema by univariable logistic analysis. *

	Presence of DR		Presence of DME	
	OR (95% CI)	P	OR (95% CI)	P
Age	1.01 (0.96–1.06)	0.621	0.98 (0.92–1.05)	0.629
Sex, male	1.05 (0.70–1.59)	0.806	0.53 (0.28–1.00)	0.051
Waist circumference, cm	0.99 (0.97–1.02)	0.620	1.03 (0.97–1.04)	0.866
Hip circumference, cm	0.98 (0.95–1.01)	0.118	1.04 (1.00–1.08)	0.072
BMI, kg/m ²	0.99 (0.95–1.03)	0.496	1.03 (0.98–1.08)	0.270
Smoking history	0.79 (0.45–1.42)	0.435	0.47 (0.16–1.35)	0.159
Drinking history	0.68 (0.35–1.34)	0.266	0.69 (0.24–2.02)	0.499
SBP, mmHg	1.01 (1.00–1.03)	0.035	1.00 (0.99–1.02)	0.645
DBP, mmHg	1.01 (0.98–1.03)	0.586	1.00 (0.97–1.03)	0.969
Hypertension, yes	0.88 (0.60–1.31)	0.529	0.95 (0.55–1.66)	0.862
HbA1C, %	1.23 (1.09–1.39)	0.001	1.43 (1.23–1.67)	<0.0001
Duration of diabetes, years	1.18 (1.31–1.23)	<0.0001	1.20 (1.14–1.27)	<0.0001
Triglyceride, mmol/L	1.04 (0.91–1.17)	0.577	0.88 (0.61–1.27)	0.495
Creatinine, μ mol/L	1.01 (0.99–1.02)	0.302	1.01 (0.99–1.03)	0.503
eGFR [#]	0.99 (0.99–1.00)	0.198	1.00 (0.99–1.01)	0.578
MAU, mg/24h	1.01 (1.01–1.02)	<0.0001	1.01 (1.01–1.02)	<0.0001
25(OH)D, nmol/L	1.00 (0.99–1.01)	0.629	0.99 (0.98–1.01)	0.391
P1NP, ng/mL	1.00 (0.99–1.01)	0.506	0.99 (0.97–1.00)	0.094
AZGP1, ng/mL [#]	1.00 (1.00–1.00)	0.987	1.00 (1.00–1.00)	0.332
FGF21, ng/mL	1.00 (0.98–1.02)	0.938	1.02 (0.99–1.04)	0.184
Osteonectin, ng/mL	1.00 (1.00–1.00)	0.133	1.00 (1.00–1.00)	0.820
β -CTX [*] , ng/mL	0.55 (0.37–0.82)	0.003	0.39 (0.22–0.69)	0.001
Fasting blood glucose, mmol/L	1.13 (1.05–1.21)	0.001	1.25 (1.15–1.36)	<0.0001
Blood glucose medication, yes	18.07 (2.34–139.49)	0.006	>999.999 (<0.001, >999.999)	0.978
Cholesterol, mmol/L	1.03 (0.89–1.20)	0.651	1.36 (1.11–1.66)	0.003

AZGP1, Zinc-alpha-2-glycoprotein; β -CTX, beta C-terminal telopeptide of collagen type I; CKD-EPI, Cockcroft-Gault equation; DBP, diastolic blood pressure; DME, diabetic macular edema; DR, diabetic retinopathy; FBG, fasting blood glucose; FGF21, fibroblast growth factor 21; HbA1C, hemoglobin A1C; MAU, microalbuminuria; PINP, procollagen type 1 N-terminal propeptide; SBP, systolic blood pressure; TG, triglyceride.

eGFR was estimated using CKD-EPI.

[#] One patient with a high level of AZGP1 was excluded from analysis.

^{*} β -CTX was log-transformed in analysis to account for skewness in the data.

Table 3Multivariable logistic analysis for association with DR and DME.[#]

Response	Predictors	OR (95% CI)	P
DR	Fasting blood glucose	1.04 (0.94–1.15)	0.456
	HbA1C	1.14 (0.95–1.38)	0.155
	MAU	1.01 (1.00–1.01)	0.088
	Duration of diabetes	1.16 (1.11–1.22)	<0.0001
	Sex, male	0.79 (0.46–1.32)	0.361
	PINP	1.01 (1.00–1.02)	0.271
DME	Fasting blood glucose	1.19 (1.04–1.37)	0.011
	HbA1C	1.22 (0.92–1.61)	0.170
	MAU	1.00 (1.00–1.01)	0.172
	β -CTX [*]	0.36 (0.13–1.02)	0.053
	Duration of diabetes	1.23 (1.15–1.32)	<0.0001
	Sex, male	0.29 (0.12–0.71)	0.006
	PINP	1.02 (0.98–1.05)	0.345
	AZGP1	1.00 (1.00–1.00)	0.223
	FGF21	0.99 (0.93–1.04)	0.607

We included variables selected by purposeful selection.

AZGP1, zinc-alpha-2-glycoprotein; β -CTX, beta C-terminal telopeptide of collagen type I; DR, diabetic retinopathy; DME, diabetic macular edema; FGF21, fibroblast growth factor 21; HbA1C, hemoglobin A1C; MAU, microalbuminuria; PINP, procollagen type 1 N-terminal propeptide.

[#] one patient with a high level of AZGP1 was excluded from analysis.

^{*} β -CTX was log-transformed in analysis to account for skewness in the data.