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Clinical Implications of Bone Metabolic Markers in Patients with Type 2 Diabetes Mellitus

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ABSTRACT This paper compared the changes of bone metabolic markers (BTM) in patients with Type 2 Diabetes Mellitus (T2DM) with or without diabetic kidney disease (DKD). 204 T2DM patients were divided into 4 groups, such that group 1 had no albuminuria, group 2 had microalbuminuria, group 3 with macroalbuminuria, and group 4 with DKD according to urine albumin and albumin to creatinine ratio (ACR) and levels of serum creatinine (SCR). The ACR, SCR, uric acid (UA), osteocalcin (OC), procollagen type I N-terminal propeptide (PINP), C-terminal telopeptide of type I collagen (CTX) and bone mineral density of the lumbar vertebrae 1-4 (BMDLV) in DKD were considerably higher than those who were non-DKD ($P<0.05$). When there is no DKD in diabetes, ACR detection is the most sensitive and specific. When it comes to DKD, the SCR is the most sensitive, and PINP specificity is greater than CTX, but the sensitivity is lower. DKD is a major contributor to osteoporosis risk.

INTRODUCTION

Type 2 diabetes mellitus (T2MD) patients' glucose and lipid metabolism are involved in the development of diabetic osteoporosis. It can cause many complications, including cardiovascular disease, kidney disease, neurological disease, retinal disease, and changes in osteocytes metabolism, affecting bone mineralisation (Räkel et al. 2008). It has been reported that the bone mineral density of diabetic patients has not changed or increased, but patients with type 1 (T1DM) and type 2 (T2DM) have a higher risk of fracture than normal people (Räkel et al. 2008). In a clinical study, the probability of fractures occurring after 10 years of T2DM was 30.6 percent, which was drastically higher than that of non-diabetic patients (Rathmann and Kostev 2015). After 2 months of high-fat or high-fat and high-sugar diet, the mice gained weight and developed insulin resistance, oxidative stress, and metabolic disorders. High-fat, high-sugar-fed mice developed high glucose and sustained high insulin after 6 months, and fatty lesions and endothelial dysfunction occurred after 8 months (Lozano et al. 2016). In the long-term, high insulin and hyperglycaemia conditions, it inhibits osteoblasts, activates osteoclasts, causes bone mineral density (BMD) to decrease, and the probability of fracture increases (Jakab et al. 2021).

Common mechanisms for increased risk of T1DM and T2DM fractures include accumulation of advanced glycation end products (AGEs) caused by intracellular hyperglycaemia, chronic hyperglycaemia, hypercalciuria with poor glycaemic control, and AGEs, which are permanently deposited sugar oxidation products (Valderrábano and Linares 2018).

Among patients with T2DM, 20.5 percent have kidney disease. diabetic kidney disease (DKD) is one of the most serious microvascular consequences associated with diabetes. The early manifestation is a slight increase in urinary albumin, which gradually progresses to a large increase in albuminuria and serum creatinine (SCR) levels. In diabetic patients with kidney disease, renal dysfunction leads to impaired activation of vitamin D in the kidneys and decreased 1, 25 dihydroxy vitamin D (VD3). VD3 deficiency not only accelerates bone turnover, increases bone loss, reduces bone density, and affects bone mass (Busse et al. 2013), but also reduces muscle mass and muscle function and increases the risk of falls, indicating that VD3 deficiency increases the risk of osteoporotic frac-

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tures through double adverse effects on bones and muscles (Järvinen et al. 2008; Ebeling 2014).

Bone turnover markers (BTMs), which include VD3, osteocalcin (OC), procollagen type I N-terminal propeptide (PINP) and C-terminal telopeptide of type I collagen (CTX), are indicators that represent bone metabolism. BTMs are bone turnover dynamics markers that have been connected to osteoporosis and fracture. A meta-analysis discovered that diabetics have lower levels of indicators for both bone formation and bone resorption, demonstrating that diabetes mellitus is a state of impaired bone metabolism (Hygum et al. 2017).

In T2DM patients, the serum bone formation markers OC and PINP are decreased, the bone resorption marker CTX is shown by some authors to be reduced but other revealed no difference (Rubin 2015).

The insulin therapy has little effect on above BTMs, metformin treatment lowers BTMs, and better glycemic control may influence bone resorption activity, glycosylated haemoglobin (HbA1c) is negatively correlated with CTX, but not with PINP (Stage et al. 2018). Although T2DM patients are correlated with total, hip, upper arm and ankle fractures (Wang et al. 2019), both increased and decreased levels of BMD have high fracture risk and the altered BMD alone may not properly account for the increased fracture risk (Miao et al. 2005).

Objective of the Study

This paper compared the changes in biological indicators and BTMs in patients with T2DM/DKD and evaluated their clinical application significance in order to establish reference intervals for the biological indicators and BTMs in T2DM patients.

MATERIAL AND METHODS

Two hundreds and four T2DM patients who were hospitalised from May 2015 to March 2018 were enrolled in this study. The inclusion criteria were that all patients meet the diagnostic criteria for T2DMs that is, HbA1c $\leq$ 6.5 percent, or fasting plasma glucose (FPG) $\leq$ 126 mg/dl (7.0 mmol/l.) The exclusion criteria were the presence of liver disease, hyperthyroidism and other endocrine and metabolic diseases, other diseases affecting calcium and phosphorus metabolism such as tumour bone metastases, serious systemic diseases, long-term use of hormones or oestrogen, and other drugs that affect bone metabolism.

The subjects were divided into four groups according to ACR and SCR (Diabetes Care 2008), namely, group 1 as the no albuminuria group (the control group) with ACR < 30 mg/g (n=77, male 38, female 39, mean age 60.64±11.09 years), group 2 as the microalbuminuria group with ACR 30-300 mg/g (n=36, male 11, female 25, mean age 65.36 ± 10.31 years), group 3 as the macroalbuminuria group with ACR > 300 mg/g, SCR <97 ìmol/l (n=5, male 24, female 21, mean age 61.56±9.69 years), and group 4, DKD group with ACR > 300 mg/g, SCR < 177 ìmol/l (n=46, male 29, female 17, mean age 64.44±10.03 years). On the second morning after fasting for 8-10 hours, the patients' venous blood was taken and various biochemical indicators were detected by Aptio Automation System (Siemens Healthcare Diagnostics and Bio-Rad Variant II HbA1C analyser (Bio-Rad, USA)) including FPG, serum levels of HbA1c, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, triglyceride (TG), total cholesterol (TC), calcium, phosphorus, SCR, uric acid (UA) and ACR. The BTMs were detected by electrochemiluminescence detection method (automated Cobas e601 analyser. Roche), including VD3. The total osteocalcin (OC, ab270202, Abcam, Cam, UK USA), PINP (ab210966, Abcam, Cam, UK) and CTX (LS-F21708, LSBio, WA, USA) levels were assayed by enzyme-linked immunosorbent assay (ELISA). Dual X-ray absorptiometry (DXA, Norland XR-800, Norland Cooper Surgical, Trumbull, Connecticut) was used to quantify (in grams) the BMD of the lumbar vertebrae 1-4 (BMDLV), femoral neck (BMDFN), Wards triangle (BMDWT) and femoral greater trochanter (BMDT). The above parameters as well as the sex, age, duration of diabetes, blood pressure and body mass index (BMI) were compared in the 4 groups. The study was approved by the First Affiliated Hospital of the University of Science and Technology of China, Anhui Provincial Hospital. Each subject provided written informed permission.

Statistical Analysis

Statistical analysis using SPSS 17.0 was performed for each biomarker. The results were presented as mean ± standard deviation (SD). The

homogeneity of variance was assessed by one-way ANOVA followed by Bonferroni test. The Chi-square test was used for testing relationships between categorical variables. The Pearson test was used for the independent data to reveal the relationships among the factors tested. Receiver operating characteristic curve (ROC) analysis was performed on the mean of systolic blood pressure (SBP), HbA1c, SCR, UA, ACR, VD3, OC, PINP, CTX and BMDLV for their sensitivity and specificity. $P<0.05$ was considered to be statistically significant.

RESULTS

The changes in BTMs and BMDs among the four groups were compared and the Pearson correlation for SCR, UA, ACR, VD3, OC, PINP and CTX were analysed.

Observation of Biological Indicators

Through the comparison of clinical data and biochemical indicators among the four groups, there were no significant differences observed in age, BMI, DBP, FPG, ALT, AST, ALP, albumin, TG, TC, calcium and phosphorus ($P>0.05$, Tables 1 and 2), but the SBP (mmHg) in groups 2, 3 and 4 (139.81±13.70, 149.16±17.75, 148.57±17.11) were significantly higher than in group 1 (128.30±13.87, $P<0.05$). Compared to groups 1, 2 and 3, the SCR (213.57±97.56 versus 73.79±12.24, 76.46±16.91, 73.32±20.63 μmol/l), UA (424.79±102.14 versus 302.37±90.34, 307.01±96.65, 327.46±104.99 μmol/l), ACR (3216.72±2444.50 versus 14.75±5.99, 81.33±74.00, 1557.35±1741.46 μg/mg), OC (32.32±18.38 versus 16.17±5.66, 17.37±10.76, 16.20±9.71 ng/ml), PINP (103.16±106.15 versus 49.16±45.30,54.78±47.38, 49.61±36.71 ng/ml), and CTX (763.50±399.97 versus 427.35±227.02, 421.68±306.42, 453.52±458.20 ng/ml) in group 4 were significantly higher (all $P<0.01$, Table 3). VD3 (ng/ml) was substantially lower in group 4 than in groups 1, 2 and 3 (7.44±5.36 versus 14.95±8.06, 12.77±5.68, 12.43±7.39) ($P<0.01$, Table 3).

Sensitivity and Specificity Analysis of Biological Indicators

The receiver operating characteristic (ROC) curve results suggested that the ACR (cut-off 28.6 μg/mg) of the area under the ROC curve [(AUC)=

Table 1: Demographics and characteristics of the patients (n = 204)

Index(Normal range)	*Group 1 n=77*	*Group 2 n=36*	*Group 3 n=45*	*Group 4 n=46*	X^2	*P*	*F*	*P*	*Bonferroni test*	
Age (y) (range)	60.64±11.09 (39-84)	65.36±10.31 (45-81)	61.56±9.69 (44-82)	64.44±10.03 (38-82)			2.36	0.07		
Sex					8.79	0.03				
Male (n)	38	11	24	29						
Female (n)	39	25	21	17						
BMI (kg/m^2)	24.70±1.26	25.45±1.94	25.95±2.18	23.96± 2.37			1.81	0.15		
SBP (mmHg)<130	128.30±13.87	139.81±13.70	149.16±17.75	148.57±17.11			21.71	0.00	**	
DBP (mmHg)<90	79.32±7.78	78.36±15.15	84.82±12.66	84.11±10.10			3.28	0.11		
FPG (mmol/l)4-5.5	8.06±3.51	9.43±3.08	9.10±3.17	8.79±4.81			1.56	0.21		
HbA1c (3.6-5.3%)	8.30±2.08	9.22±1.60	8.96±1.94	7.82±1.87			6.15	0.00	#	
Duration of type 2 diabetes (y), (range)	9.05±6.63 (0.04-26)	13.38±6.61 1 (0.5-35)	12.01±6.27 (0.5-24)	13.36±7.40 1 (0.03-28)			5.26	0.00	*	

Table 2: Biological indicators with no significant differences in the four groups of patients (n=204)

Index (Normal range)	*Group 1 n=77*	*Group 2 n=36*	*Group 3 n=45*	*Group 4 n=46*	*F*	*P*	*Bonferroni test*
Serum ALT (8-56 IU/l)	24.58±24.47	21.14± 13.98	21.19±11.50	19.89±10.87	0.58	0.68	
Serum AST (6-34 IU/l)	21.98±13.311	22.06± 13.38	20.44± 7.17	21.62± 5.80	0.11	0.96	
Serum ALP (42-128 IU/l)	84.31±30.98	83.81± 31.48	76.28±19.72	88.95± 29.33	1.7	0.17	
Serum albumin (35-55g/dl)	39.96±3.20	40.12± 3.20	37.72± 6.44	34.88± 5.86	0.57	0.63	
Serum TG (<1.7 mmol/l)	1.70±1.00	2.02± 1.47	2.20± 1.25	2.23± 1.54	2.61	0.053	
Serum TC (3-5.2 mmol/l)	4.19±0.90	6.66± 14.91	4.91± 1.24	5.16± 1.37	1.32	0.27	
Serum calcium (2.1-2.5mmol/l)	2.22±0.13	2.25± 0.13	2.21± 0.16	2.20± 0.17	0.485	0.69	
Serum phosphorus (0.8-1.5mmol/l)	1.21±0.20	1.14± 0.14	1.18± 0.19	1.25± 0.21	2.018	0.113	

ALT, alanine transaminase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; TG, total cholesterol; TC, triglyceride

Table 3: Biological indicators with significant changes in the four groups of patients (n=204)

Index (Normal range)	*Group 1 n=77*	*Group 2 n=36*	*Group 3 n=45*	*Group 4 n=46*	*F*	*P*	*Bonferroni test*
Serum creatinine (45-110 µmol/l)	73.79± 12.24	76.46± 16.91	73.32± 20.63	213.57± 97.56	88.96	0.00	*
Serum uric acid (142-416 µmol/l)	302.37± 90.34	307.01± 96.65	327.46± 104.99	424.79± 102.14	15.01	0.00	*
ACR (2.7-26.10 µg/mg)	14.75± 5.99	81.33± 74.00	1557.35± 1741.46	3216.72±2444.50	56.86	0.00	*/**
Serum VD3 (30~56.45 ng/ml)	14.95± 8.06	12.77± 5.68	12.43± 7.39	7.44± 5.36	10.73	0.00	*
Osteocalcin(4-10 ng/ml)	16.17± 5.66	17.37± 10.76	16.20± 9.71	32.32± 18.38	20.19	0.00	*
PINP (8.36-654.50 ng/ml)	49.16± 45.30	54.78± 47.38	49.61± 36.71	103.16± 106.15	7.20	0.00	*
CTX (0.04~1.90 ng/ml)	427.35±227.02	421.68±306.42	453.52± 458.20	763.50± 399.97	9.52	0.00	

ACR, urine albumin and albumin to creatinine ratio; VD3, 1, 25 dihydroxy vitamin D; PINP, procollagen type I N-terminal propeptide; CTX, C-telopeptide fragments of collagen type I a1 chains;
*: group 4 compared to groups 1, 2 and 3, P<0.01.
**: group 3 compared to groups 1 and 2, P<0.01.

1.00] test was most sensitive and specific in patients with T2DM without albuminuria (Fig. 1). Group 4 showed that SCR (cut-off 113 μmol/l, AUC=1.00), ACR (cut-off 526 μg/mg, AUC=0.93) and OC (cut-off 20.8 ng/ml, AUC=0.78) were slightly better than PINP (cut-off 53.3 ng/ml, AUC=0.77) and CTX (cut-off 749 ng/ml, AUC=0.76), but OC was less sensitive (0.80) than CTX (0.91) (0.91) (Fig. 2). VD3 levels (cut-off 5.01 ng/ml, AUC=0.76) were a sensitive indicator (sensitivity=0.91), but specificity (0.51) was lower than other indicators (Table 4).

Comparison of Bone Mineral Density Results

There were no significant changes in BDMFN (g/cm^2), that is, 0.86±0.17 in group 1, 0.83±0.18 in group 2, 0.90±0.19 in group 3 and 0.87±0.14 in group 4, in BDMWT (g/cm^2), that is, 0.75±0.22 in group 1, 0.72±0.23 in group 2, 0.75±0.24 in group 3 and 0.72±0.19 in group 4, and in BDMT (g/cm^2), that is, 0.77±0.17 in group 1, 0.72±0.15 in group 2, 0.77±0.17 in group 3 and 0.76±0.13 in group 4, but BDMLV (1.13±0.18 g/cm^2) in group 4 was significantly higher than those of group 1 (0.99±0.18 g/cm^2) and group 2 (1.03±0.18 g/cm^2) ($P<0.05$, Table 5). BMDLV (cut-off 1.01 g/cm^2) was not a sensitive index (sensitivity=0.54, specificity=0.81) in reflecting the levels of BTMs in DKD patients (Tables 4 and 5).

In general, ALP was not statistically different among the 4 groups. However, the parameters of SCR (213.57±97.56 μmol/l), UA (424.79±102.14 μmol/l), ACR (3216.72±2444.50 μg/mg), OC (32.32±18.38ng/ml), PINP (103.16±106.15 ng/ml), CTX(763.50±399.97 ng/ml) and BMDLV (1.13±0.18 g/cm^2) in group 4 were considerably higher than those of non-DKD ($P<0.05$, Tables 3 to 5).

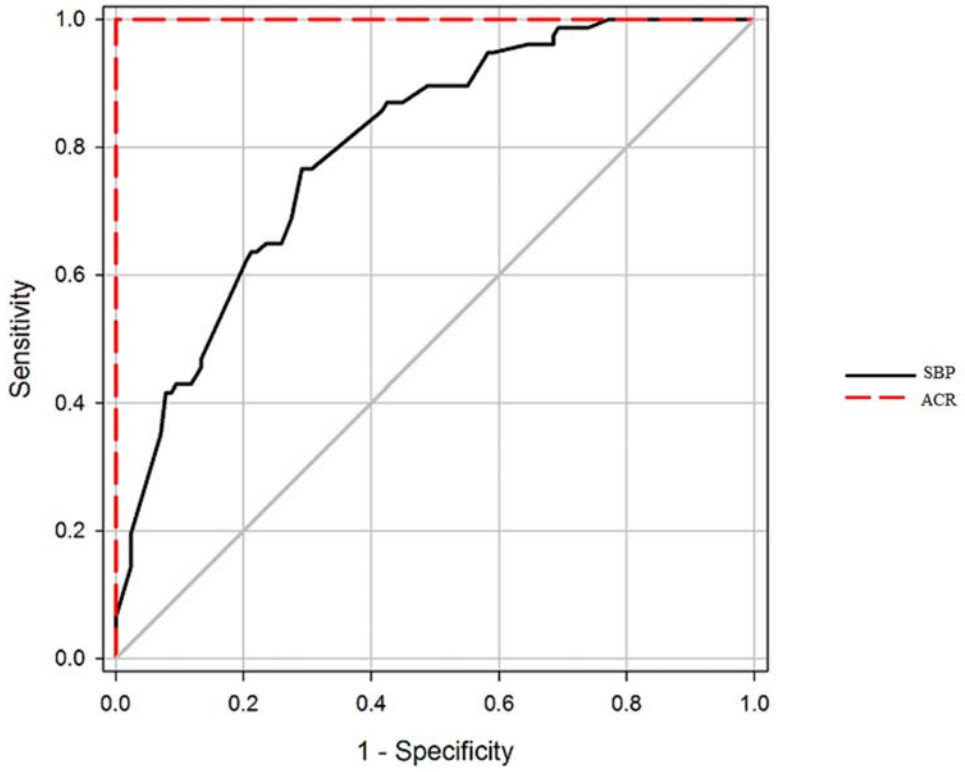

Fig. 1. Receiver operating characteristic curve plots of systolic blood pressure (SBP) and urine albumin and albumin to creatinine ratio (ACR) for patients with and without albuminuria

Table 4: Receiver operating characteristic curve analysis

Index	*No albuminuria / microalbuminuria*				*Macroalbuminuria / DKD*			
	Cutoff value	*AUC*	*Sensitivity*	*Specificity*	*Cutoff value*	*AUC*	*Sensitivity*	*Specificity*
SBP (mmHg)	139	0.79	0.76	0.69	149	0.69	0.79	0.51
HbAlc (%)	9.85	0.55	0.88	0.27	8.35	0.68	0.51	0.77
Serum creatinine (µmol/l)	90.5	0.70	0.93	0.51	113	1.00	1.00	1.00
Serum uric acid (µmol/l)	302	0.63	0.53	0.69	305	0.79	0.52	0.95
ACR (µg/mg)	28.6	1.00	1.00	1.00	526	0.93	0.83	0.95
VD3 (ng/ml)	7.62	0.66	0.85	0.40	5.01	0.76	0.91	0.51
Osteocalcin (ng/ml)	20.8	0.58	0.84	0.42	20.8	0.78	0.80	0.70
PINP (ng/ml)	72.5	0.59	0.93	0.25	53.3	0.77	0.73	0.70
CTX (ng/ml)	724	0.56	0.92	0.25	749	0.76	0.91	0.49
BMD of Lumbar vertebra 1-4 (g/cm^2)	1.02	0.65	0.64	0.60	1.01	0.68	0.54	0.81

DKD, diabetic kidney disease; AUC, area under receiver operating characteristic curve curve; SBP, systolic blood pressure; HbA1c, glycosylated hemoglobin; ACR, urine albumin and albumin to creatinine ratio; VD3, 1, 25 dihydroxy vitamin D; PINP, procollagen type I N-terminal propeptide; CTX, C-terminal telopeptide of type I collagen; BMD, BMD, bone mineral density.

Table 5: BMD changes in the four groups of patients (n=204)

Index	*Group 1 n=77*	*Group 2 n=36*	*Group 3 n=45*	*Group 4 n=46*	*F*	*P*	*Bonferroni test*
BMD of lumbar vertebra 1-4 (g/cm^2)	0.99±0.18	1.03±0.18	1.08±0.18	1.13±0.18	6.75	0.00	#
BMD of femoral neck (g/cm^2)	0.86±0.17	0.83±0.18	0.90±0.19	0.87±0.14	1.54	0.21	
BMD of Wards triangle (g/cm^2)	0.75±0.22	0.72±0.23	0.75±0.24	0.72±0.19	1.17	0.32	
BMD of femoral greater trochanter (g/cm^2)	0.77±0.17	0.72±0.15	0.77±0.17	0.76±0.13	1.05	0.37	

BMD, bone mineral density

#: group 4 compared to groups 1 and 2, P<0.05

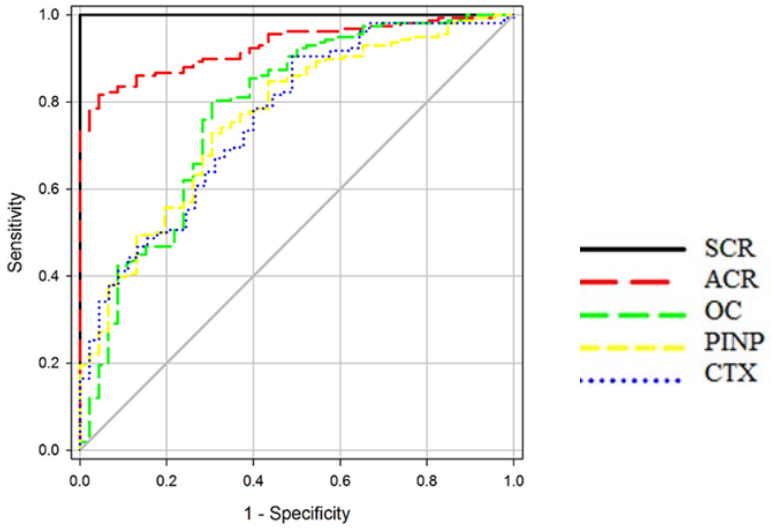

Fig. 2. Receiver operating characteristic curve plots of creatinine (SCR), urine albumin and albumin to creatinine ratio ACR, osteocalcin (OC), procollagen type I N-terminal propeptide (PINP) and C-terminal telopeptide of type I collagen (CTX) for patients with and without Diabetic kidney disease

Pearson Correlation Analysis of Biological Indicators

Correlation analysis (Table 6) showed that the concentration of OC was significantly positive correlated with ACR of group 2 (r=0.56, P<0.01), PINP of groups 2, 3 and 4 (r=0.56, 0.73 and 0.57, respectively, all P<0.01), and CTX of groups 2, 3 and 4 (r=0.83, 0.72 and 0.76, respectively, all P<0.01). That of VD3 was negatively correlated with ACR [-0.50 (P<0.01)] in group 4. The evaluation of the 5 major indices, including SCR, ACR, OC, PINP and CTX make contributions to the prevention of osteoporosis at the stage of DKD (Table 6).

No significant correlation was observed for each pair of SCR, UA, ACR, VD3, OC, PINP and CTX in group 1. In groups 2, 3 and 4, OC was positively correlated with CTX, with correlation coefficients of 0.83, 0.72 and 0.76 (p<0.01), respectively, and OC was positively correlated with PINP with correlation coefficients of 0.56, 0.73, 0.57 (p<0.01), respectively, but the positive correlation between OC and ACR was only observed in group 2 with a correlation coefficient of 0.56 (p<0.01). Once the patient has macroalbuminuria or DKD, this correlation between OC and ACR no longer existed (Table 6). In group 3, a significant correlation was found between PINP and CTX with correlation coefficients of 0.66 (P<0.01). In group 4, significant correlations were found between SCR and BTMs with correlation coefficients of 0.61 (P<0.01) for OC, 0.56 for PINP (P<0.01), and 0.48 for CTX (p<0.05) (Table 6).

DISCUSSION

According to a recent study, in diabetes, BTMs correlate with the urine albumin to creatinine ratio, which is an indicator of early-stage nephropathy. The urine ACR was adversely linked with BMD at every place tested (femoral neck, trochanter, inner hip, Ward's triangle, total hip,and lumbar vertebrae). Urine ACR was positively linked with osteocalcin, CTX and PINP. VD3 was found to be inversely associated to urine ACR. A multiple regression research that accounted for age, BMI, disease duration, and other clinical parameters found no significant relationship between ACR and BMD levels or CTX. BMD changes and bone transformation acceleration can occur in the early stages of diabetic nephropathy, and bone transformation acceleration can occur before BMD alterations. As a result, monitoring bone metabolism indicators in T2DM patients in the early stages is crucial (Zhao et al. 2019).

Table 6: Pearson correlation analysis data

	SCR	*UA*	*ACR*	*VD3*	*Osteocalcin*	*PINP*	*CTX*
Group 1							
SCR	1.00						
UA	0.41	1.00					
ACR	-0.24	-0.08	1.00				
VD3	0.01	-0.20	-0.19	1.00			
Osteocalcin	-0.11	-0.10	0.17	-0.03	1.00		
PINP	0.10	0.09	-0.11	-0.03	0.08	1.00	
CTX	0.06	-0.19	-0.05	-0.02	0.25	0.03	1.00
Group 2							
SCR	1.00						
UA	0.39	1.00					
ACR	0.01	0.00	1.00				
VD3	0.27	-0.08	0.13	1.00			
Osteocalcin	0.03	0.07	0.56**	0.41	1.00		
PINP	0.06	0.20	0.28	0.13	0.56**	1.00	
CTX	-0.15	0.09	0.48	0.20	0.83**	0.39	1.00
Group 3							
SCR	1.00						
UA	0.23	1.00					
ACR	0.36	0.08	1.00				
VD3	-0.08	0.12	-0.42	1.00			
Osteocalcin	0.26	0.04	-0.13	-0.01	1.00		
PINP	0.13	0.02	0.07	-0.07	0.73**	1.00	
CTX	-0.02	0.05	0.00	-0.10	0.72**	0.66**	1.00
Group 4							
SCR	1.00						
UA	0.25	1.00					
ACR	0.24	-0.31	1.00				
VD3	-0.15	0.28	-0.50**	1.00			
Osteocalcin	0.61**	0.14	0.37	-0.04	1.00		
PINP	0.56**	-0.06	0.41	-0.24	0.57**	1.00	
CTX	0.48*	-0.04	0.30	-0.03	0.76**	0.43	1.00

SCR, serum creatinine; UA, uric acid; ACR, urine albumin and albumin to creatinine ratio; VD3, 1, 25 dihydroxy vitamin D; PINP, procollagen type I N-terminal propeptide CTX, C-terminal telopeptide of type I collagen. * Indicated P<0.05; **Indicated P<0.01.

The age, DBP, BMI, FPG, ALT, AST, ALP, albumin, TG, TC, calcium and phosphorus showed no significant differences among the groups (P>0.05). The gender, SBP, HbA1c and duration of diabetes were different among the groups (P<0.05, Tables 1 and 2), which was the shortest (9.05±6.63 years) in group 1 and the longest (13.36±7.40 years) in DKD. The levels of VD3 decreased in patients with DKD. The serum level of SCR, UA, ACR, OC, PINP and CTX in DKD was higher than that in groups 1, 2 and 3 (Table 3, P<0.05).

In this study, the serum OC and PINP of the group 4 were higher than those of the groups 1-3, suggesting that the renal injury of diabetic nephropathy began to show an increase in osteoblast activity and showed a high conversion type.

The content of OC, PNIP in the blood circulation mainly reflects the bone turnover. The serum OC and PNIP of the group 4 were higher than groups 1, 2 and 3, suggesting that their activity osteoblasts was increased and the formation of new bone was increased.

In group 4, elevated serum CTX reflected an increase in osteoclast activity and bone loss. However, the level of VD was significantly lower in group 4 than in the other three groups, indicating a decrease in VD3. A decrease in the level of VD3 resulted in a decrease in intestinal calcium absorption, and low blood calcium stimulated the secretion of parathyroid hormone (PTH), which in turn promoted bone resorption of osteoclasts, leading to osteoporosis. However, the concentration of serum calcium and phosphorus did not reflect the dynamic changes of osteogenic and osteoclasts.

Some research have identified an inverse connection between HbA1c levels and blood VD3 lev-

els (Kostoglou-Athanassiou et al. 2013), while others have discovered that VD3 supplements improve glucose management in T2DM patients (Kostoglou-Athanassiou et al. 2013; Mitri et al. 2011). Physiologically, vitamin D appears to promote insulin receptor expression. As a result, VD3 deficiency may be linked to insulin resistance (Mathieu et al. 2006). The effects of VD3 on osteoporosis have been controversial. VD3 was not associated with osteoporosis in multiple clinical analyses. However, another study found that VD3 could increase the levels of calcium and phosphorus in the blood, while reducing the excessive secretion of PTH (Lips and van Schoor 2011).

Diabetes was linked to total, hip, upper arm and ankle fractures. Furthermore, T1DM patients had a higher incidence of total, hip, and ankle fractures than T2DM patients (Wang et al. 2019). This study discovered that BMDLV was significantly higher in DKD patients than in groups 1 and 2, suggesting that it was a useful estimate of the bone fragility of diabetic patients. Dennison et al. believe that T2DM patients with insulin resistance in the body, a large amount of insulin through the receptors on osteoblasts, is conducive to bone formation and increased bone density. However, due to lack of insulin, the bone absorption is greater than the formation, which eventually leads to a decrease in bone density and osteoporosis (Dennison et al. 2004; Yamamoto and Sugimoto 2016).

AGEs are closely related to the pathogenesis of this unique clinical outcome through physical and biological effects on the deterioration of the material properties of bone (Yamamoto and Sugimoto 2016).

Blood pressure (BP) control is important in preventing stroke, cardiovascular disease, and albuminuria. Many studies have shown that BP control is reno-protective (Berlowitz et al. 2017). The UK prospective diabetes study suggested that a 10-mmHg decrease in SBP reduced diabetic microvascular complications and DKD (Adler et al. 2000). SBP decreased 5.6 mmHg reduced the development of microalbuminuria significantly (Patel et al. 2007).

The researchers' data also confirmed that monitoring SBP was more sensitive and easier than monitoring diastolic blood pressure (DBP) in individuals presenting albuminuria, but its specificity decreased from 0.69 to 0.51 in patients with DKD. Among patients with T2DM, the most sensitive indicator for distinguishing between albuminuria free and albuminuria was serum ACR concentration [AUC=1.00, cut-off 28.6 (μg/mg)], followed by SBP and SCR. With SBP >139 mmHg and SCR >90.5 μmol/l, most individuals developed albuminuria. The most sensitive indicator for distinguishing between DKD-free and DKD ass SCR level [AUC=1.00, cut-off 113 (μmol/l)], followed by ACR, UA, OC, PINP, CTX and VD and the cut-off values were 526 μg/mg, 305 μmol/l, 20.8 ng/ml, 53.3 ng/ml, 749 ng/ml and 5.01 ng/ml, respectively.

The most common metabolic bone condition is osteoporosis, which is characterised by structural degeneration of bone structure and an increased risk of fracture. Typically, bone material strength and bone biomechanical quality in T2DM patients was abnormal (Farr and Khosla 2016). An increase in bone turnover leads to a deterioration of the bone microstructure, which in addition to low BMD leads to an increased risk of fracture (Follet et al. 2004; Banse et al. 2002), but CTX, which was not predictive (Chapurlat et al. 2000).

ALP is a membrane-bound tetrameric enzyme found in the plasma membrane of osteoblasts, showing an association with a bone remodelling activity, particularly in Paget disease (Migliorini et al. 2021). It has an important role in osteoid formation and mineralisation by enzymatic degradation of the inhibitor of mineralisation, pyrophosphate at an alkaline pH (Rader 2017) and ALP was the first BTM to be used in clinical and research settings. Several isomers of ALP have been identified in liver, intestine, placenta and bone (Shetty et al. 2016).

OC is a 49-amino-acid calcium-binding peptide released by mature osteoblasts; indeed, OC concentrations coincide with direct assessments of bone growth by histomorphometry (Oury et al. 2011; Ferron et al. 2010), but it is a beneficial BTM in steroid-induced osteoporosis due to its short half-life (Clemens and Karsenty 2001).

IOF advocated PINP as a reference bone formation marker because of its low intra-individual variability, smaller circadian variation, room temperature stability, and acceptable assay precision (Vasikaran et al. 2011).

CTX comes in two forms: isomerized and non-isomerized, and it is released during bone resorption. To limit this preanalytical variability, it is advisable to collect the sample in the morning after the overnight fast to diminish physiological cir-

cumstances such as growing children and pathological situations such as malignant bone illnesses (Garnero et al. 1997; Garnero et al. 2008; Clowes et al. 2002).

BMD measurement is the current gold standard test for diagnosing osteoporosis (Meeta et al. 2013), yet BMD levels exceeding the WHO definition of osteoporosis are seen in around half of women who sustain osteoporotic fractures (Nguyen et al. 2007).

Individuals' peak bone mass and rate of bone loss are influenced by genetic, epigenetic, and environmental variables (Mitchell and Yerges-Armstrong 2011). Studies have shown ethnicity-based variations in the distribution of BTMs and the need for the establishment of ethnicity-specific reference ranges for each BTM for clinical use in different populations, region-specific risk entities, lower dietary calcium intake and Vitamin D deficiency, physical activity and drugs, and so on (de Papp et al. 2007; Ardawi et al. 2010; Szulc et al. 2013).

Overall, prospective studies examining the link between bone formation markers and eventual fracture risk have failed to demonstrate that anabolic BTMs are useful for this purpose (Garnero et al. 1996). When a change in the level of a bone marker is noticed in an individual patient, it must be evaluated in the context of the marker's variability, which includes fasting and food intake. Preanalytical and analytical variability, ethnic variances, and the lack of an ethnicity-based reference interval for each group are all major drawbacks of BTMs.

CONCLUSION

Serum ACR detection is the most sensitive and specific when there is no DKD in diabetes. With DKD, the SCR is the most sensitive, PINP specificity is better than CTX, but the sensitivity is lower than CTX. VD3 is a sensitivity (0.91) indicator, but specificity is lower than SCR and ACR. DKD-OC levels are not specific indicators of osteoporosis and BMDs cannot reflect VD3 level changes. DKD is an important risk factor for osteoporosis.

RECOMMENDATIONS

In order to prevent and treat diabetes with osteoporosis, early control of hyperglycaemia should be actively recommended to prevent and treat complications, especially diabetic nephropathy.

ABBREVIATIONS LISTS

T2MD: type 2 diabetes mellitus
BMD: bone mineral density
DKD: diabetic kidney disease
SCR: serum creatinine
VD3: 1, 25 dihydroxy vitamin D
OC: osteocalcin
PINP: procollagen type I N-terminal propeptide
CTX: C-terminal telopeptide of type I collagen
HbA1c: Glycosylated haemoglobin
FPG: fasting plasma glucose
ACR: albumin to creatinine ratio
BMI: body mass index
ALT: alanine aminotransferase
AST: aspartate aminotransferase
ALP: alkaline phosphatase
TG: triglyceride
TC: total cholesterol
UA: uric acid
DXA: dual X-ray absorptiometry
BMDLV: BMD of the lumbar vertebrae 1-4
BMDFN: femoral neck
BMDWT: Ward's triangle
BMDT: femoral greater trochanter
ROC: receiver operating characteristic curve
AUC: area under ROC curve

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study was approved by the First Affiliated Hospital of University of Science and Technology of China Anhui Provincial Hospital. Written informed consent was obtained from each participant.

AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

COMPETING INTERESTS

There are no potential conflicts of interest to disclose.

AUTHORS' CONTRIBUTIONS

Yujie Wu is responsible to the guarantor of integrity of the entire study, study concepts, literature research, data and statistical analysis, experimental studies, clinical studies, data acquisition, manuscript preparation, editing and review. The author read and approved the final manuscript.

REFERENCES

Adler AI, Stratton IM, Neil HA, Yudkin JS, Matthews DR, Cull CA, Wright AD, Turner RC, Holman RR 2000. Association of systolic blood pressure with macrovascular and microvascular complications of type 2 diabetes (UKPDS 36): Prospective observational study. *BMJ,* 321: 412–419.

Ardawi MS, Maimani AA, Bahksh TA, Rouzi AA, Qari MH, Raddadi RM 2010. Reference intervals of biochemical bone turnover markers for Saudi Arabian women: A cross-sectional study. *Bone,* 47: 804–814.

Banse X, Sims TJ, Bailey AJ 2002. Mechanical properties of adult vertebral cancellous bone: Correlation with collagen intermolecular cross-links. *J Bone Miner Res,* 17: 1621–1628.

Berlowitz DR, Foy CG, Kazis LE, Bolin LP, Conroy MB, Fitzpatrick P, Gure TR, Kimmel PL, Kirchner K, Morisky DE, Newman J, Olney C, Oparil S, Pajewski NM, Powell J, Ramsey T, Simmons DL, Snyder J, Supiano MA, Weiner DE, Whittle J; SPRINT Research Group 2017. Effect of intensive blood-Pressure treatment on patient-reported outcomes. *N Engl J Med,* 377(8): 733-744.

Busse B, Bale HA, Zimmermann EA, Panganiban B, Barth HD, Carriero A, Vettorazzi E, Zustin J, Hahn M, Ager JW 3rd, Püschel K, Amling M, Ritchie RO 2013. Vitamin D deficiency induces early signs of aging in human bone, increasing the risk of fracture. *Sci Transl Med,* 5: 188r–193r.

Chapurlat RD, Garnero P, Bréart G, Meunier PJ, Delmas PD 2000. Serum type I collagen breakdown product (serum CTX) predicts hip fracture risk in elderly women: The EPIDOS study. *Bone,* 27: 283–286.

Clemens TL, Karsenty G 2001. The osteoblast: An insulin target cell controlling glucose homeostasis. *J Bone Miner Res,* 26: 677–680.

Clowes JA, Hannon RA, Yap TS, Hoyle NR, Blumsohn A, Eastell R 2002. Effect of feeding on bone turnover markers and its impact on biological variability of measurements. *Bone,* 30: 886–890.

Diabetes Care 2008. 39, S1–112. From <http://diabetesjournals.org/content/39/Supplement_1.toc.> (Retrieved on 22 September 2020).

Dennison EM, Syddall HE, Aihie Sayer A, Craighead S, Phillips DI, Cooper C 2004. Type 2 diabetes mellitus is associated with increased axial bone density in men and women from the Hertfordshire Cohort Study: Evidence for an indirect effect of insulin resistance? *Diabetologia,* 47: 1963-1968.

de Papp AE, Bone HG, Caulfield MP, Kagan R, Buinewicz A, Chen E, Rosenberg E, Reitz RE 2007. A cross-sectional study of bone turnover markers in healthy premenopausal women. *Bone,* 40: 1222–1230.

Ebeling PR 2014. Vitamin D and bone health: Epidemiologic studies. *Bonekey Rep,* 3: 511.

Farr JN, Khosla S 2016. Determinants of bone strength and quality in diabetes mellitus in humans. *Bone,* 82: 28–34.

Ferron M, Wei J, Yoshizawa T, Del Fattore A, DePinho RA, Teti A, Ducy P, Karsenty G 2010. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. *Cell,* 142: 296–308.

Follet H, Boivin G, Rumelhart C, Meunier PJ 2004. The degree of mineralization is a determinant of bone strength: A study on human calcanei. *Bone,* 34: 783–789.

Garnero P, Hausherr E, Chapuy MC, Marcelli C, Grandjean H, Muller C, Cormier C, Bréart G, Meunier PJ, Delmas PD 1996. Markers of bone resorption predict hip fracture in elderly women: The EPIDOS prospective study. *J Bone Miner Res,* 11: 1531-1538.

Garnero P, Fledelius C, Gineyts E, Serre CM, Vignot E, Delmas PD 1997. Decreased beta-isomerization of the C-terminal telopeptide of type I collagen alpha 1 chain in Paget's disease of bone. *J Bone Miner Res,* 12: 1407–1415.

Garnero P, Bauer DC, Mareau E, Bilezikian JP, Greenspan SL, Rosen C, Black D 2008. Effects of PTH and alendronate on type I collagen isomerization in postmenopausal women with osteoporosis: The PaTH study. *J Bone Miner Res,* 23: 1442–1448.

Hygum K, Starup-Linde J, Harsløf T, Vestergaard P, Langdahl BL 2017. Mechanisms in endocrinology: Diabetes mellitus, a state of low bone turnover - a systematic review and meta-analysis. *European Journal of Endocrinology,* 176(3): R137–R157.

Jakab J, Miškiæ B, Mikšiæ Š, Juraniæ B, Æosiæ V, Schwarz D, Vèev A 2021. Adipogenesis as a potential anti-obesity target: A review of pharmacological treatment and natural products. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 14: 67–83.

Järvinen TL, Sievänen H, Khan KM, Heinonen A, Kannus P 2008. Shifting the focus in fracture prevention from osteoporosis to falls. *BMJ,* 336: 124–126.

Kostoglou-Athanassiou I, Athanassiou P, Gkountouvas A, Kaldrymides P 2013. Vitamin D and glycemic control in diabetes mellitus type 2. *Ther Adv Endocrinol Metab,* 4: 122–128.

Lips P, van Schoor NM 2011. The effect of vitamin D on bone and osteoporosis. *Best Pract Res Clin Endocrinol Metab,* 25: 585-591.

Lozano I, Van der Werf R, Bietiger W, Seyfritz E, Peronet C, Pinget M, Jeandidier N, Maillard E, Marchioni E, Sigrist S, Dal S 2016. High-fructose and high-fat diet-induced disorders in rats: Impact on diabetes risk, hepatic and vascular complications. *Nutr Metab (Lond),* 13: 5.

Mathieu C, Gysemans C, Giulietti A, Bouillon R 2006. Vitamin D and diabetes. *Diabetologia,* 48: 1247–1257.

Meeta, Digumarti L, Agarwal N, Vaze N, Shah R, Malik S 2013. Clinical practice guidelines on menopause: An

executive summary and recommendations. *J Midlife Health,* 4: 77–106.

Miao J, Brismar K, Nyrén O, Ugarph-Morawski A, Ye W 2005. Elevated hip fracture risk in type 1 diabetic patients: A population-based cohort study in Sweden. *Diabetes Care,* 28: 2850–2855.

Migliorini F, Maffulli N, Spiezia F, Tingart M, Maria PG, Riccardo G 2021. Biomarkers as therapy monitoring for postmenopausal osteoporosis: A systematic review. *Journal of Orthopaedic Surgery and Research,* 16(1): 318.

Mitchell BD, Yerges-Armstrong LM 2011. The genetics of bone loss: Challenges and prospects. *J Clin Endocrinol Metab,* 96: 1258–1268.

Mitri J, Muraru MD, Pittas AG 2011. Vitamin D and type 2 diabetes: A systematic review. *Eur J Clin Nutr,* 65: 1005–1015.

Nguyen ND, Eisman JA, Center JR, Nguyen TV 2007. Risk factors for fracture in nonosteoporotic men and women. *J Clin Endocrinol Metab,* 92: 955–962.

Oury F, Sumara G, Sumara O, Ferron M, Chang H, Smith CE, Hermo L, Suarez S, Roth BL, Ducy P, Karsenty G 2011. Endocrine regulation of male fertility by the skeleton. *Cell,* 144: 796–809.

Patel A, ADVANCE Collaborative Group, MacMahon S, Chalmers J, Neal B, Woodward M, Billot L, Harrap S, Poulter N, Marre M, Cooper M, Glasziou P, Grobbee DE, Hamet P, Heller S, Liu LS, Mancia G, Mogensen CE, Pan CY, Rodgers A, Williams B 2007. Effects of a fixed combination of perindopril and indapamide on macrovascular and microvascular outcomes in patients with type 2 diabetes mellitus (the ADVANCE trial): A randomised controlled trial. *Lancet,* 370: 829–840.

Rader BA 2017. Alkaline phosphatase, an unconventional immune protein. *Frontiers in Immunology,* 8: 897.

Räkel A, Sheehy O, Rahme E, Lelorier J 2008. Osteoporosis among patients with type 1 and type 2 diabetes. *Diabetes Metab,* 34: 193-205.

Rathmann W, Kostev K 2015. Fracture risk in patients with newly diagnosed type 2 diabetes: A retrospective database analysis in primary care. *J Diabetes Complications,* 29: 766-770.

Rubin MR 2015. Bone cells and bone turnover in diabetes mellitus. *Curr Osteoporos Rep,* 13: 186–191.

Stage TB, Christensen MH, Jørgensen NR, Beck-Nielsen H, Brøsen K, Gram J, Frost M 2018. Effects of metformin, rosiglitazone and insulin on bone metabolism in patients with type 2 diabetes. *Bone,* 112: 35-41.

Shetty S, Kapoor N, Bondu JD, Thomas N, Paul TV 2016. Bone turnover markers: Emerging tool in the management of osteoporosis. *Indian J Endocrinol Metab,* 20(6): 846-852.

Szulc P, Bauer DC, Estell R 2013. Biochemical markers of bone turnover in osteoporosis. In: CJ Rosen (Editor-in-Chief), R Bouillon (Senior Associate Editor), JE Compston (Senior Associate Editor), V Rosen (Senior Associate Editor): *Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism.* Chapter 35. 8th Edition. Wiley-Blackwell: UK. 1104. ISBN 978-1-118-45388-9, pp. 297–302.

Valderrábano RJ, Linares MI 2018. Diabetes mellitus and bone health: Epidemiology, etiology and implications for fracture risk stratification. *Clin Diabetes Endocrinol,* 4: 9.

Vasikaran S, Eastell R, Bruyère O, Foldes AJ, Garnero P, Griesmacher A, McClung M, Morris HA, Silverman S, Trenti T, Wahl DA, Cooper C, Kanis JA, IOF-IFCC Bone Marker Standards Working Group 2011. Markers of bone turnover for the prediction of fracture risk and monitoring of osteoporosis treatment: A need for international reference standards. *Osteoporos Int,* 22: 391–420.

Wang H, Ba Y, Xing Q, Du JL 2019. Diabetes mellitus and the risk of fractures at specific sites: A meta-analysis. *BMJ Open,* 9: e024067.

Yamamoto M, Sugimoto T 2016. Advanced glycation end products, diabetes, and bone strength. *Curr Osteoporos Rep,* 14: 320-326.

Zhao X, Zhang XM, Yuan N, Yu XF, Ji LN 2019. Associations of bone mineral density and bone metabolism indices with urine albumin to creatinine Ratio in Chinese patients with Type 2 diabetes. *Experimental and Clinical Endocrinology and Diabetes,* 6(1): 50–55.

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