THE EXPRESSIONS OF PINP, β-CTX AND sICAM-1 IN TYPE 2 DIABETES MELLITUS AND MICROVASCULAR DISEASE

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ABSTRACT

Objective: *The purpose of this study is to investigate the expressions of PINP, β-CTX and sICAM-1 in Type 2 diabetes mellitus and microvascular disease.*

Methods: *A total of 315 patients with type 2 diabetes mellitus (T2DM) who were recruited from January 2015 to December 2016 were included in this study. The subjects were randomly assigned to either the control group or the observation group. Each group included patients with or without retinopathy and/or microalbuminuria. The general data of the patients' height and weight were recorded in detail. An automatic biochemical analyser was used to determine fasting glucose, blood calcium and other biochemical indicators. Parathyroid hormone, glycosylated haemoglobin and other indicators were measured by radioimmunoassay. The levels of PINP, β-CTX and sICAM-1 were determined by an ELISA.*

Results: *Compared with the control group, the expressions of HbA1C, TC, TG, Cr, BUN, UA, PTH, β-CTX, and sICAM-1 in the observation group were significantly increased ($p<0.05$), whereas the 25(OH)D_3 level was obviously lower ($p<0.05$). For the DN, DR and DN/DR groups, the levels of PINP and sICAM-1 in the DN/DR group were significantly higher than those in the control group ($P<0.01$). The levels of β-CTX in the DR and DN/DR groups were significantly higher than those in the control group ($P<0.01$). The levels of β-CTX and sICAM-1 in the DN group were significantly lower than those in the DN/DR group ($P<0.01$). In the control group, β-CTX expression was negatively correlated with BUN ($\gamma=-5.586$, $P=0.003$), Cr ($\gamma=-6.159$, $P=0.000$) and UA ($\gamma=-3.087$, $P=0.038$). In the observation group, PINP was negatively correlated with BUN ($\gamma=-0.357$, $P=0.016$) and UA ($\gamma=-0.063$, $P=0.041$). β-CTX was negatively correlated with BUN ($\gamma=-0.456$, $P=0.008$), Cr ($\gamma=-6.122$, $P=0.000$), UA ($\gamma=-5.568$, $P=0.000$) and 25(OH)D_3 ($\gamma=-6.897$, $P=0.000$). Moreover, PINP was positively correlated with HbA1C ($\gamma=3.016$, $P=0.019$) and PTH ($\gamma=6.973$, $P=0.000$). sICAM-1 was positively correlated with LDL ($\gamma=0.486$, $P=0.007$).*

Conclusions: *The β-CTX and sICAM-1 expression levels can be used as predictors to monitor the early risk stratification in T2DM and microvascular disease.*

Keywords: *Type 2 diabetes mellitus, microangiopathy, PINP, β-CTX, sICAM-1.*

DOI: 10.19193/0393-6384_2019_2_173

Received *November 30, 2018;* ***Accepted*** *January 20, 2019*

Introduction

Type 2 diabetes mellitus (T2DM) is a common metabolic disorder that leads to calcium and phosphorus metabolic disorders through basic and endocrine metabolic changes. In particular, the risk of fracture increases significantly. More importantly, T2DM and microvascular disease have become very relevant risk factors for death and disability[(1)]. However, the relationship between T2DM and osteoporosis remains largely obscure. Recent data indicate that T2DM induces increased bone mass via regulating osteocytes[(2)]. In addition, the bone turnover markers are metabolites and enzymes secreted by bone cells, which can reflect bone remodelling. However, bone turnover may also be affected by diabetic complications. It has been reported that PINP and β-CTX are promising specific markers

for the dynamic monitoring of bone metabolism. Meanwhile, T2DM is a natural immune and cytokine-mediated inflammatory disease. The level of inflammatory markers in T2DM will increase significantly with the condition of the disease. Inflammatory factors (e.g., sICAM-1) involved in the inflammatory response and the relationship with T2DM and microvascular disease has become a popular issue worldwide[4-5]. Thus, we performed this study to investigate the expression levels of PINP, β-CTX and sICAM-1 in T2DM and microvascular disease.

Materials and methods

General Information

Participants were recruited from January 2015 to October 12 to obtain a total of 315 patients with T2DM. The following inclusion criteria were used[6]: Females aged less than 45 years old and premenopausal and males less than 55 years old; T2DM consistent with the classification and diagnostic criteria revised by the WHO in 1999; and the diagnostic criteria of T2DM combined with vascular diseases are based on the Guidelines for the Prevention and Treatment of Type 2 Diabetes in China (2013 Edition). The following exclusion criteria were used[7]: Patients with type 1 diabetes and related complications; patients with primary liver and kidney diseases; patients with infectious diseases; infectious diseases; patients with rheumatic immune diseases; patients on long-term bed rest; patients with other endocrine diseases; patients with a family history of osteoporosis; recent use of drugs affecting bone metabolism; and patients with recent fractures. The cases were approved by the Hospital Ethics Committee, and the patients signed informed consent. The patients were divided into two groups: 170 patients without retinopathy and/or microalbuminuria served as the control group, and 145 patients with retinopathy and/or microalbuminuria served as the observation group. These patients included 117 females and 198 males.

Methods

General data on age, sex, medication and history of diabetes were obtained. The body mass index (BMI) was calculated as body weight (kg)/height^2 (m^2). Diastolic blood pressure (DBP) and systolic blood pressure (SBP) were recorded in the patients at rest. Five-millilitre fasting blood samples were collected after overnight fasting. Blood calcium (Ca), fasting blood glucose (FBG), high density lipoprotein (HDL), total cholesterol (TC), low density lipoprotein (LDL), uric acid (UA), triglyceride (TG), creatinine (Cr), blood urea nitrogen (BUN) and other biochemical indexes were measured with an automatic biochemical analyser. APS fundus angiography was used to check the fundus microangiopathy. The levels of PINP, β-CTX and sICAM-1 were measured with an ELISA. A radioimmunoassay was used to determine the glycosylated haemoglobin (HbA1c), parathyroid hormone (PTH), 25 hydroxyvitamin D3 ((25 OH) D3), and fasting C peptide (FCP) levels. The ratio of urinary albumin to creatinine (ACR) was measured by special protein dry type immunoscattering chromatography.

Outcome Measurement

The following classifications were used: large amount of albuminuria: ACR more than 300 μg/mg. Microalbuminuria: 30 μg/mg<ACR≤299 μg/mg. Normal range: ACR≤30 μg/mg. Poor glycaemic control: HbA1c≥7%. Hypertension: using hypertension drugs or BP≥140/90 mmHg. Obesity: BMI>28 kg/m2. Dyslipidaemia: TG>2.26 mmol/L, HDL<1.4 mmol/L and/or LDL≥4.14 mmol/L, using lipid-lowering drugs.

Statistical Analysis

All data were analysed with IBM SPSS Statistics 22.0. The general measurement data were expressed by the mean ± standard deviation ($\bar{x} \pm s$). Comparisons between two groups were performed by the t test or Mann-Whitney U test, and comparisons between multiple groups were performed by a one-way ANOVA. Enumeration data were expressed as the percentage (%), and the chi-square test was used for comparisons. The Pearson correlation was used for the correlation analysis, and $P<0.05$ was considered statistically significant.

Results

Adverse effects and general information in each group

There were no significant differences in age, sex, BMI, obesity, history of disease, dyslipidaemia, history of hypertension, SBP, DBP, and poor blood glucose control between the two groups ($P > 0.05$), as shown in Table 1.

Components	Observation group (n=145)	Control group (n=170)	t/χ^2	*P*
Age	46.85±5.46	45.99±7.21	1.203	0.229
Female (premenopausal)	52 (35.86)	65 (38.24)	0.189	0.664
Male	93 (64.14)	105 (61.76)		
BMI (kg/ m^2)	25.12±3.42	25.31±3.22	0.507	0.612
Fat	56 (38.62)	64 (37.65)	0.032	0.859
Family history of diabetes (years)	9.78±7.21	9.12±3.56	-0.287	0.774
Blood fat	94 (64.83)	99 (58.24)	0.924	0.336
High blood pressure	106 (73.10)	118 (69.41)	0.519	0.472
SBP (mmHg)	147.24±22.57	145.67±17.13	0.686	0.493
DBP (mmHg)	81.23±10.23	80.32±9.76	0.8037	0.422
Combined microalbuminuria	101 (69.67)	0	/	/
Combined retinopathy	88 (60.69)	0	/	/
Poor glycaemic control	96 (66.21)	103 (60.59)	1.062	0.303

Table. 1: Factors affecting diabetes mellitus and the baseline characteristics of the studied participants.

The expressions of PINP, β-CTX, and sICAM-1 and the comparison of biochemical indicators

The level of PINP in the observation group was higher than that in the control group, but the difference was not statistically significant (P>0.05). The levels of HBA1C, TC, TG, Cr, BUN, UA, PTH, β-CTX, and sICAM-1 in the observation group were significantly higher than those in the control group (P<0.05). The level of 25 (OH) D3 in the observation group was lower than that in the control group, and the difference was statistically significant (P<0.05), as shown in Table 2.

Components	Observation group (n=145)	Control group (n=170)	*Z/t*	*P*
FBG (mmol/l)	10.34±3.57	10.11±3.78	0.552	0.581
HbA1C (%)	9.78±2.56	8.45±1.56	5.452	<0.001
TC (mmol/l)	5.23±1.62	4.89±1.23	2.069	0.039
TG (mmol/l)	2.45±2.12	1.87±1.45	-3.169	0.002
FCP (pmol/ml)	0.46±0.12	0.43±0.13	2.115	0.035
HDL (mmol/l)	1.34±0.21	1.21±0.34	3.999	0.000
LDL (mmol/l)	2.88±1.03	2.86±0.99	0.175	0.861
VLDL (mmol/l)	1.22±0.73	0.92±0.67	-2.78	0.007
Cr (μmol/l)	68.72±15.45	60.78±15.76	4.497	<0.001
BUN (mmol/l)	5.89±1.75	4.89±1.76	5.039	<0.001
UA (μmol/l)	307.13±77.56	277.78±70.71	3.511	0.001
Ca (mmol/l)	2.45±0.13	2.65±0.67	-3.809	<0.001
PTH (pg/ml)	29.67±11.67	23.89±10.68	4.587	<0.001
25 (OH) D_3 (ng/ml)	14.11±3.98	16.77±4.87	-5.333	<0.001
PINP (ng/ml)	47.56±16.21	46.13±13.24	0.848	0.396
β-CTX (ng/ml)	0.53±0.43	0.42±0.26	-1.992	0.023
sICAM-1 (μg/L)	442.36±64.56	213.32±55.43	33.879	<0.001

Table. 2: The expressions of PINP, β-CTX, and sICAM-1 and a comparison of biochemical indicators.

A comparison of PINP, β-CTX and sICAM-1 between genders

The levels of PINP, β-CTX and sICAM-1 were lower in males than in premenopausal women, but the difference was not statistically significant (P>0.05), as shown in Table 3.

Components	Male (n=198)	Female (n=117)	*Z/t*	*P*
PINP (ng/ml)	48.21±14.56	49.56±15.54	0.775	0.439
β-CTX (ng/ml)	0.45±0.24	0.47±0.23	-0.151	0.440
sICAM-1(μg/L)	443.81±83.67	444.12±82.89	0.032	0.975

Table. 3: Comparison of PINP, β-CTX and sICAM-1 levels between genders.

Comparison of PINP, β-CTX and sICAM-1 levels in different types of microangiopathy

To compare the levels of PINP, β-CTX, and sICAM-1 between different types of microangiopathy groups, the observation group was divided into the diabetic nephropathy group (DN), the diabetic retinopathy group (DR), and the diabetic retinopathy with nephropathy group (DN/DR). The levels of PINP and sICAM-1 in the DN/DR group were significantly higher than those in the control group (P<0.01). The levels of β-CTX in the DR and DN/DR groups were significantly higher than those in the control group (P < 0.01). The β-CTX and sICAM-1 levels in the DN group were significantly lower than those in the DN/DR group (P<0.01), as shown in Table 4.

Components	Number of cases	PINP (ng/ml)	β-CTX (ng/ml)	sICAM-1 (μg/L)
Control group	170	35.12±7.45	0.39±0.11	215.67±54.78
Diabetic nephropathy group (DN)	58	38.35±8.38*	0.48±0.09*	218.15±56.34
Diabetic retinopathy group (DR)	48	39.42±7.56*	0.51±0.08*	221.43±56.13
Diabetic retinopathy complicated with nephropathy (DN/DR)	39	53.67±8.19$^{*\#\&}$	0.68±0.11$^{*\#\&}$	593.81±63.56$^{*\#\&}$
F		60.97	90.86	593.81
P		<0.001	<0.001	<0.001

Table. 4: Comparison of PINP, -CTX and sICAM-1 levels in different types of microangiopathy.

Note: Compared to the control group, $^{*}P<0.05$. Compared to the DN group, $^{\#}P<0.05$. Compared to the DR group, $^{\&}P<0.05$.

The correlation analysis of two groups of PINP, β-CTX and sICAM-1 with each parameter

In the control group, β-CTX was negatively correlated with BUN (γ=-5.586, P=0.003), Cr (γ=-6.159, P=0.000) and UA (γ=-3.087, P=0.038). In the observation group, PINP was negatively correlated with BUN (γ=-0.357, P=0.016) and UA (γ=-0.063, P=0.041). β-CTX was negatively correlated with BUN (γ=-0.456, P=0.008), Cr (γ=-6.122, P=0.000), UA (γ=-5.568, P=0.000) and 25 (OH) D3 (γ=-6.897, P=0.000), whereas it was positively correlated with HbA1C (γ=3.016, P=0.019) and PTH (γ=6.973, P=0.000). sICAM-1 was positively correlated with LDL (γ=0.486, P=0.007), as shown in Table 5.

Components	Observation group (n=145)						Control group (n=170)					
	PINP		β-CTX		sICAM-1		PINP		β-CTX		sICAM-1	
	γ	P	γ	P	γ	P	γ	P	γ	P	γ	P
SBP (mmHg)	0.078	0.437	0.167	0.178	0.056	0.423	-0.064	0.432	0.145	0.112	0.156	0.312
DBP (mmHg)	0.172	0.301	-0.086	0.423	0.167	0.276	0.076	0.401	0.176	0.321	0.132	0.175
FBG (mmol/l)	0.073	0.512	-0.002	0.967	0.065	0.512	-0.078	0.427	0.023	0.756	-0.076	0.421
HbA1C (%)	-0.043	0.678	3.016	0.019	-0.021	0.543	-0.002	0.856	-0.123	0.356	-0.142	0.243
TC (mmol/l)	-0.189	0.265	-0.076	0.531	-0.231	0.125	-0.165	0.125	0.067	0.434	-0.242	0.113
TG (mmol/l)	-0.086	0.423	-0.278	0.102	-0.043	0.231	-0.134	0.234	0.054	0.578	-0.253	0.162
FCP (pmol/ml)	-0.068	0.482	-0.143	0.378	-0.047	0.362	-0.067	0.523	-0.069	0.367	-0.001	0.761
HDL (mmol/l)	0.152	0.178	-0.124	0.287	0.148	0.176	-0.217	0.167	0.043	0.604	0.231	0.154
LDL (mmol/l)	0.082	0.437	-0.113	0.325	0.486	0.007	-0.057	0.567	-0.034	0.675	0.342	0.137
VLDL (mmol/l)	0.074	0.513	-0.204	0.105	0.045	0.387	-0.078	0.367	-0.167	0.145	0.124	0.324
Cr (μmol/l)	-0.021	0.865	-6.122	0.000	0.156	0.361	0.054	0.621	-6.159	0.000	-0.154	0.165
BUN (mmol/l)	-0.357	0.016	-0.456	0.008	0.134	0.262	-0.167	0.123	-5.586	0.003	-0.023	0.769
UA (μmol/l)	-0.063	0.041	-5.568	0.000	-0.232	0.273	-0.078	0.324	-3.087	0.038	0.132	0.142
Ca (mmol/l)	0.115	0.321	-6.897	0.000	0.142	0.351	0.176	0.125	0.013	0.876	0.023	0.756
PTH (pg/ml)	0.152	0.178	6.973	0.000	0.171	0.266	0.023	0.765	0.093	0.942	0.075	0.657
$25(OH)D_3$ (ng/ml)	-0.167	0.124	-0.712	0.028	-0.232	0.152	-0.143	0.167	-0.132	0.265	-0.241	0.175

Table. 5: Correlation analysis of PINP, β-CTX and sICAM-1 with each parameter in each group.

Discussion

Microangiopathy is a specific disease of diabetes mellitus that includes retinopathy, nephropathy, cardiomyopathy, etc.[8]. Diabetic retinopathy (DR) has become one of the four leading causes of blindness, and diabetic nephropathy (DN) is the leading cause of end-stage renal disease in western countries. β-CTX and PINP are specific substances in bone cell metabolism that can be used as independent predictors of osteoporotic fracture and osteoporosis. As a marker of bone formation, the increased expression of PINP indicates an accelerated rate of collagen I synthesis, changes in collagen I and an accelerated process of bone formation[9]. Ghonaim M et al.[10] found that the level of PINP in patients with T2DM and microangiopathy was significantly higher than that in the control group. β-CTX is recommended as an international guideline for bone resorption; changes in its level can reflect bone resorption sensitively. However, diabetic complications may affect the process of bone turnover.

T2DM is an immune inflammatory response mediated by many factors, and inflammation is the trigger of the origin of insulin resistance. One of the biomarkers of vascular endothelial injury is sICAM-1. Vascular endothelial cell injury and the disturbance of glucose metabolism are factors related to increased serum sICAM-1 levels in T2DM patients. The mechanism of elevated serum sICAM-1 levels is associated with adhesion between

endothelial cells and leukocytes, which leads to the accumulation of extracellular matrix, injury of endothelial cells, increase of vascular permeability, platelet aggregation and adhesion, resulting in angiopathy and atherosclerosis[11-13].

Many factors such as gender, age, obesity and menopause affect the bone metabolism of diabetic patients[14]. In this study, the subjects were divided into premenopausal women and males to avoid interference. The results showed that there was no significant difference in age, sex, BMI, obesity, hypertension history and poor blood glucose control between the two groups (P>0.05). The levels of PINP, β-CTX, and sICAM-1 were lower in males than in premenopausal women, but the difference was not statistically significant (P>0.05); therefore, the above factors were excluded in the analysis. Moreover, the PINP level of the observation group was higher than that of the control group, but the difference was not statistically significant (P>0.05). The levels of β-CTX and sICAM-1 in the observation group were significantly higher than those in the control group (P<0.05), which suggests that increased bone turnover rate in patients with T2DM and microangiopathy leads to bone loss. Additionally, increased sICAM-1 levels induced endothelial cell injury and accelerated the inflammatory response. The level of 25 (OH) D3 in the observation group was significantly lower than that in the control group (P<0.05), suggesting that the expression of 25 (OH) D3 may affect the level of bone turnover markers. The levels of PINP and sICAM-1 in the DN/DR group were significantly higher than those in the control group (P<0.01). The levels of β-CTX in the DR and DN/DR groups were significantly higher than those in the control group (P<0.01). The levels of β-CTX and sICAM-1 in the DN group were significantly lower than those in the DN/DR group (P<0.05). These results were consistent with those in Liu C's study[15-16]. In addition, the results showed that β-CTX was negatively correlated with BUN, UA, Cr, and 25 (OH) D3 in the observation group but was positively correlated with PTH, which suggests that β-CTX expression may be related to PTH. Increased blood concentration of PTH could enhance bone resorption and activate osteoclast activity. The negative correlation between PINP and BUN indicates that 25 (OH) D3 deficiency and renal dysfunction may affect the levels of PINP and β-CTX. The positive correlation between β-CTX and HbA1C indicates that poor blood glucose control was associated with β-CTX levels in patients with T2DM microangiopathy. The positive correlation between sICAM-1 and LDL showed that the change in LDL was correlated with the expression of sICAM-1 in patients with T2DM microangiopathy.

In conclusion, β-CTX and sICAM-1 levels can be a good indicator for monitoring patients with T2DM complicated with microangiopathy.

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