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Serum dickkopf1 (DKK1), bone metabolism and atherosclerotic disease in patients with type 2 diabetes

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Summary

Background and objectives: Type 2 diabetes (T2DM) is a risk factor for osteoporotic fractures and cardiovascular disease. The aims of our study were to evaluate serum Dickkopf-1(DKK1) levels in a cohort of T2DM patients and to analyze its relationships with bone metabolism and atheroesclerotic disease (AD).

Patients and methods: We studied 126 subjects: T2DM patients (n: 72, mean age 58,2±6 years) and non-diabetic subjects (n: 54, mean age 55,4±7 years). DKK-1 was measured by enzyme-linked immunosorbent assay (ELISA, Biomedica Gruppe). Bone mineral density (BMD) was measured by dual-energy X-ray absorptiometry (DXA). The presence of AD (cerebrovascular disease, peripheral arterial disease, ischemic heart disease) was recorded. Intima-media thickness (IMT) was determined by doppler ultra- sonography and aortic calcification by evaluation of lateral view conventional X-rays.

Results: We did not find significant differences in DKK1 between groups. Serum DKK1 concentrations were significantly higher in females in total sample (24,3±15,2 vs 19,6±10,2 pmol/L, p=0,046) and in T2DM group (27,5±17,2 vs 19,8±8,9 pmol/L, p=0,025). There was a positive correlation between serum DKK1 and LS BMD in total sample (r=0,183, p=0,048). However, we did not find a significant relationship with osteoporosis diagnosis or morphometric vertebral fractures. Serum DKK1 was significantly higher in T2DM patients with AD (26,4±14,5 pmol/L vs 19,1±11,6 pmol/L, p=0,026) and also in patients with abnormal IMT (26,4±15,1 pmol/L vs 19,8±11,3 pmol/L, p=0,038). In the ROC curve analysis to evaluate the usefulness of DKK-1 as a marker for high risk of AD, the area under the curve was 0,667 (95% confidence interval: 0,538-0,795; p=0,016). A concentration of 17,3 pmol/L or higher showed a sensitivity of 71,4% and a specificity of 60% to identify an increased risk of AD.

Conclusions: Circulating DKK1 levels are higher in T2DM with AD and are associated with an abnormal IMT in this cross-sectional study. DKK1 may be involved in vascular disease of T2DM patients.

Key words: *serum Dickkopf1, bone metabolism, atherosclerotic disease, type 2 diabetes mellitus.*

Introduction

Type 2 diabetes mellitus (T2DM) has been linked to an increased risk of fractures at any site despite increased bone mineral density (BMD)[1,2]. Furthermore, atherosclerosis is the major pathogenic mechanism in patients with diabetic macrovascular disease related to a thickening of the arterial wall, the development of the atheromatous plaque and vascular calcification[3]. Atherosclerotic vascular disease is more common in patients with osteoporosis, suggesting several common pathophysiological pathways[4]. In addition, epidemiological data support a relationship between low BMD and the presence of advanced atherosclerotic disease in T2DM[5,6].

Wnt signaling pathways are involved in various physiological processes including cell and tissue differentiation along with morphogenesis of organs[7]. The discovery of the Wnt signaling pathway and its relevance in bone homeostasis has contributed to a better understanding of the cellular and molecular mechanisms of bone biology8. This pathway activation results in expansion of osteo-progenitor cells and a reduction in osteoblast apoptosis, leading to anabolic effects on bones[8]. The canonical Wnt pathway is regulated by multiple families of antagonists, such as Dickkopf-1 (DKK1). DKK1 regulates Wnt signaling by binding to a co-receptor, linked to low density lipoprotein (LRP) 5/6. Furthermore, DKK1 binds to other molecules, such as Kremen transmembrane proteins, to increase its inhibitory activity of the Wnt pathway[9]. The relationship between serum DKK1 and bone mass has been analyzed with contradictory data[10-12].

Given the inverse relationship between bone fragility and atherosclerosis, the role of the Wnt signaling pathway in the process of atherosclerosis is being researched. In preclinical studies, the Wnt signaling pathways are involved in the process of vascular calcification[13], inflammation[14], monocyte adhesion and trans-endothelial migration[15]. There are also recent data that show a relationship between serum levels of DKK1 and atherosclerosis in humans[16,17].

In this context, the objectives of our study were to evaluate serum DKK1 levels in a cohort of patients with T2DM and analyze its relationship with bone metabolism and atherosclerotic disease. In addition, serum DKK1 concentrations in T2DM and non-diabetic subjects were compared.

Patients and methods

Study Population

Our cross-sectional study included 126 subjects: a group of 72 T2DM patients diagnosed with diabetes according to the criteria of the American Diabetes Association (ADA, 2005) and a control group of 54 non-diabetic subjects consecutively recruited from the general population randomly in the same time period.

All study subjects met the following inclusion criteria: Caucasians, outpatient, aged between 35 and 65 and normal blood count, creatinine, liver function, calcium and phosphorus. Exclusion criteria were chronic disease except T2DM situations and treatment with drugs affecting bone metabolism. Those diabetic patients treated with thiazolidinedione were also excluded.

T2DM patients were classified into two groups according to the presence or absence of atherosclerotic disease (AD): AD group and the non- AD group, respectively. Inclusion criteria for patients with atherosclerotic disease were: cerebrovascular disease (ischemic stroke or transient ischemic attack); coronary heart disease (previous myocardial infarction, diagnosis of stable or unstable angina or coronary revascularization) or peripheral arterial disease.

The study was conducted with the approval of the Ethics Committee of the Hospital and adjusted to the relevant guidelines for human research. All patients gave informed consent to be included.

Clinical evaluation

For all patients, height, weight and waist circumference at baseline were measured according to standard procedures. BMI was calculated by dividing weight by the square of height (kg/m^2).

Blood pressure was taken in a standardized way. After 5 minutes of resting blood pressure was measured twice using a standard mercury sphygmomanometer (12 cm long, 35 cm wide). The average of the two values was used for analysis. Hypertension was defined at values ≥140/90 mmHg and/or antihypertensive treatment.

Participants reported their consumption of alcohol, tobacco and level of physical activity in a specific health questionnaire.

Analytical determinations

The plasma glucose level (GBP), glycosylated hemoglobin (HbA1c), calcium, phosphorus and serum creatinine were measured using automated laboratory techniques. High density lipoprotein (HDL), low density lipoproteins (LDL) and triglycerides were measured by standard biochemical methods. Dyslipidemia was defined as the 3rd Report of the Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (ATP-III) or had treatment with statins.

The 25-hydroxyvitamin D (25-OH-D; RIA Kit: DiaSorin, Stillwater, Minnesota, USA); serum parathyroid hormone (Roche Diagnostics SL, Barcelona, Spain immunoassay PTH) were determined.

Markers of bone formation remodeling collected were osteocalcin (OC; RIA Kit, DiaSorin, Stillwater, Minnesota, USA) and bone alkaline phosphatase (BAP; assay enzyme-linked immunosorbent - ELISA-, Tandem-R Ostase TM, Hybritech Europe, Liège, Belgium). Resorption markers included 5β tartrate-resistant acid phosphatase (TRAP5β; colorimetry, Hitachi 704 Boehringer Mannheim GmbH) and carboxy-terminal telopeptide of type 1 collagen (CTX; enzyme immunoassay analyzer CrossLaps Elecsys, Roche Diagnostics SL, Barcelona, Spain).

Serum levels of DKK1 were measured by ELISA (Biomedica Medizinprodukte GmbH and Co. KG, Vienna, Austria) according to manufacturer's instructions. The Biomedical DKK-1 ELISA (BI-20412) detects DKK-1 free. The intra-assay and interassay variability were 7% and 9% respectively. DKK1 measurement is expressed in picomoles per liter (pmol/L).

BMD and vertebral radiographs

BMD of the lumbar spine (CL) L2-L4, femoral neck (CF) and total hip (CT) was determined in all patients by dual X-ray absorptiometry (DXA) using a Hologic QDR 4500 (Whatman, MA; variation coefficient <1%). All measurements were made by the same operator. We use the criteria of the World Health Organization (WHO) for the diagnosis of osteoporosis. plain radiography X-thoracic and lumbar spine for the analysis of vertebral fractures (VF) morphometric rays was also performed, and interpreted according to the algorithm developed by McCloskey et al.[18].

Measurements of intima-media thickness and aortic calcification

The thickness of the carotid intima-media (IMT) was measured by Doppler ultrasound (TOSHIBA Vision 6000) in both carotid about 10 mm proximal to the carotid bifurcation (BIF) with 7.5 MHz probe mode B. The determination He performed by the same observer in all subjects. 10 measurements were performed on each carotid calculating the average for each artery and in turn the average of the two. It is expressed in millimeters, and pathological ≥0,9 mm GIM if defined, and the presence of atherosclerosis if GIM plate ≥1,2 mm or above 50% of adjacent GIM[19].

The presence of aortic calcification was evaluated by radiology simple lateral thoracic and lumbar spine (T4-L5)[20].

Statistical analysis

Statistical analysis of data was carried out using SPSS (version 18.0, Chicago, USA). For continuous variables, it was assessed whether these followed a normal distribution using the Kolmogorov-Smirnov test. Measures of central tendency (mean) and dispersion (standard deviation -SD-, range) were used for continuous variables, and distribution of absolute and relative frequencies for categorical variables. The differences for the variables of interest between comparison groups were performed using Student's t test for independent samples and the test of Mann-Whitney in the case of continuous variables. For categorical variables, the Pearson's chi square and Fisher's exact test were used. The relationship between quantitative variables was analyzed using bivariate correlation test of Pearson or Spearman. The usefulness of serum DKK1 as a marker of high risk for atherosclerotic disease in T2DM was analyzed using a ROC curve (receiver operating characteristic). All statistical tests were double tail. A $p<0.05$ was considered statistically significant.

Results

The clinical characteristics of the study population are summarized in Table 1.

No significant DKK1 differences between the two groups were found: T2DM, 23.35±13.78 pmol/L vs nondiabetic 11.86±20.1 pmol/L, p=0.163. Serum concentrations of DKK1 were significantly higher in women in the total sample (24.3±15.2 vs 19.6±10.2 pmol/L, p=0.046) and in the T2DM group (27.5±17.2 vs 19.8±8.9 pmol/L, p=0.025).

For bone metabolism, there was a positive correlation between DKK1 and lumbar BMD in the total sample (r=0.183; p=0.048). However, no differences depending on the diagnosis of osteoporosis or presence of morphometric vertebral fractures were found. There was also no relation to calciotropic hormones and markers of bone remodeling.

Table 2 shows AD data listed in the groups studied.

DKK1 values were significantly higher in patients with T2DM and AD (26.4±14.5 pmol/L vs 19.1±11.6 pmol/L, p=0.026) and in patients with abnormal GIM (26.4±15.1 pmol/L vs 19.8±11.3 pmol/L, p=0.038).

In the analysis of the ROC curve to evaluate DKK1's utility as a marker of high AD risk, the area under the curve was 0.667 (confidence interval - IC 95%: 0.538 to 0.795; p=0.016). A concentration of 17.3 pmol/L or higher showed a sensitivity of 71.4% and a specificity of 60% for identifying increased AD risk.

Discussion

There are few studies on the relationship between DKK1 and bone metabolism in T2DM. Our results showed higher DKK1 levels in diabetic patients with AD and pathological IMT. These findings suggest that serum DKK1 may be a predictor of the presence of atherosclerotic disease in this population. However, our data showed no differences in DKK1 between diabetic and non-diabetic subjects. For bone metabolism, serum DKK1 significant relationship with bone mineral density was found, while there was none with bone turnover markers, diagnosis of osteoporosis or the presence of morphometric vertebral fractures.

No previous work focused on evaluating the differences in serum DKK1 in accordance with the presence of diabetes. For our part, we found no differences in DKK1 in patients with T2DM and patients without diabetes. These results contrast with our previous data showing high concentrations of sclerostin in this group of diabetic patients[21]. However, the relationship between sclerostin and DKK1 has not been clearly established[22]. Unlike our previous results on sclerostin, women had higher concentrations of DKK1 in both the total sample and in the group of T2DM. The estradiol and progesterone regulate Wnt pathways in endometrial tissue[23] and the brain[24], so the effects induced by sex steroids could explain the gender differences in DKK1.

As for the relationship between bone metabolism and DKK1 we found only a weak correlation with BMD at the lumbar spine in the total sample, and no relationship with markers of bone remodeling, osteoporosis or morphometric vertebral fractures. Furthermore, the lumbar BMD can be affected by aortic calcification. Our findings confirm previous data showing no connection between DKK1 and markers of bone turnover in postmenopausal osteoporosis patients[11] and hemodialysis[12]. The association between DKK1 and BMD is not fully accepted. No relationship was found with BMD in Afro-American diabetic patients[17]. However, an inverse relationship between DKK1 and BMD and higher concentrations of DKK1 in patients with osteoporosis[10] and chronic kidney disease has been reported[22]. Therefore, data on serum DKK1 and bone metabolism are controversial, and prevent drawing clear conclusions.

In our study, the highest levels of DKK1 higher concentrations of DKK1 were related to a diabetic IMT disease were positively related to atherosclerotic disease in patients with type 2 diabetes regardless of the presence of other vascular risk factors. These results are consistent with previous data showing the relationship between vascular disease and DKK1. Patients with cerebrovascular disease have higher serum DKK1 about controls[25] and serum DKK1 correlated with calcification of the coronary arteries and atherosclerotic coronary plaques[16]. Previously, Ueland et al.[26] showed DKK1 gene expression in atherosclerotic carotid plaques and DKK1 is a new mediator of endothelial cell activation mediated by platelets. In contrast to our results, DKK1 concentrations were negatively associated with atherosclerotic plaque in black patients with T2DM[17]. As the authors note, African Americans have a lower prevalence of vascular calcification, and show opposite relationship between arterial calcification and serum concentrations of vitamin D compared to Europeans[27], which could explain the discrepancy of results.

Our study has some limitations as the cross-sectional design does not allow us to establish a cause-effect, and the sample size is relatively small and may affect the finding other interesting results.

In short, DKK1plasma concentrations did not differ according to the presence of diabetes, and we found no relationship with bone turnover markers, diagnosis of osteoporosis or the presence of morphometric vertebral fractures. However, circulating levels of DKK1 are higher in diabetic patients with atherosclerotic disease and related to a pathological IMT. These findings suggest that DKK1 may be involved in the development of atherosclerotic disease in patients with T2DM.

Conflict of interest: The authors declared no conflict of interest.

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Table 1. Characteristics of the study sample

	Total sample (n=126)	Group T2DM (n=72)	No group T2DM (n=54)	Value p
Age (years)	57±6	58±6	55±7	0.018
Male/female (n)	62/64	39/33	25/29	0.472
Clinic history				
Diabetes duration (years)	-	13.7±7.6	-	
Hypertension (%)	53.2	80.6	46.3	<0.001
Dyslipidemia (%)	65.9	94.4	70.4	<0.001
Tobacco (%)	15.1	16.7	13	0.623
Alcohol (%)	8.7	6.9	11.1	0.104
Sedentary (%)	47.6	55.6	37	0.048
Clinical evaluation				
BMI (kg/m^2)	30.5±5.9	31.4±5.7	29.3±5.9	0.043
Waist (cm)	102.6±12.4	106.4±11.4	97.4±11.9	<0.001
SBP (mm Hg)	130±20	134±97	124±17	0.002
DBP (mm Hg)	80±13	80±12	79±15	0.705
Analytical data				
GBP (mg/dL)	137.2±61.9	173±60.1	89.4±10.4	<0.001
HbA1c (%)	6.7±2.2	8±1.9	4.8±0.4	<0.001
Creatinine (mg/dL)	0.88±0.18	0.89±0.19	0.86±0.16	0.266
Calcium (mg/dL)	9.5±0.5	9.6±0.5	9.3±0.4	0.001
Phosphorus (mg/dL)	3.6±0.5	3.7±0.5	3.5±0.5	0.01
PTH (pg/mL)	43.6±19.5	38.5±18.4	50.4±19.1	<0.001
25(OH)D (ng/mL)	19.5±11.3	17.8±11.5	21.6±10.9	0.06
OC (ng/mL)	1.5±1.3	1.5±1.3	1.5±1.2	0.939
BOP (µg/L)	14±6.5	14.7±6.2	13±6.8	0.162
CTX (ng/mL)	0.266±0.155	0.209±0.132	0.338±0.153	<0.001
TRAP5b (UI/L)	1.6±0.9	1.4±1	1.8±0.8	0.02
Triglycerides (mg/dl)	142±121	169.9±149.8	104.9±47.7	<0.001
HDL (mg/dl)	53.5±15.5	49±16	59.5±12.5	<0.001
LDL (mg/dl)	111.7±35.5	96.9±34.1	130.8±27.4	<0.001
DKK1 (pg/ml)	**21.95±13.1**	**23.35±13.78**	**20.1±11.86**	0.163
DXA parameters and fractures				
BMD LS (g/cm^2)	0.977±0.148	0.954±0.146	1±0.148	0.068
BMD FN (g/cm^2)	0.820±0.124	0.817±0.132	0.823±0.117	0.792
BMD TH (g/cm^2)	0.906±0.135	0.903±0.145	0.911±0.125	0.772
T-score LS	-1.08±1.36	-1.3±1.3	0.82±1.3	0.058
T-score FN	-0.55±1.01	-0.6±1.04	-0.49±0.99	0.565
T-score TH	-0.55±0.98	-0.62±1	-0.51±0.92	0.557
Osteoporosis (%)	15.9	24.6	9.4	0.047
Fractures (%)	23	30.3	20	0.274

BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; GBP: basal plasma glucose; HbA1c: glycated hemoglobin; PTH: parathyroid hormone; 25 (OH) D: 25-hydroxyvitamin D; OC: osteocalcin; BOP: bone alkaline phosphatase; CTX: carboxy-terminal telopeptide of type 1 collagen; TRAP5b: 5β tartrate resistant acid phosphatase; HDL: high density lipoprotein; LDL: low density lipoprotein: BMD: bone mineral density; LS: lumbar spine; FN: femoral neck; TH: total hip; IMT: intima-media thickness.

Table 2. Atherosclerotic disease according to study groups

	Total sample (n=126)	Group T2DM (n=72)	No group T2DM (n=54)	Value p
Atherosclerotic disease	35.7	58.3	5.6	<0.001
Cerebrovascular disease (%)	11.9	19.4	1.9	0.002
Cardiopaty (%)	23.8	38.9	3.7	<0.001
Peripheral arterial disease (%)	7.9	13.9	0	0.005
Pathological IMT(%)	35.7	54.2	11.1	<0.001
Carotid plaque (%)	15.9	29.4	0	<0.001
Aortic calcification (%)	19	34.8	2.2	<0.001

IMT: intima-media thickness.

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