

Original Article

Correlation analysis of urine proteins and inflammatory cytokines with osteoporosis in patients with diabetic nephropathy

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Abstract

Objectives: To analyze the relationship of urine proteins and inflammatory cytokines with osteoporosis in patients with diabetic nephropathy. **Methods:** 76 patients with diabetic nephropathy in Xintai Affiliated Hospital of Taishan Medical University were selected and divided into the combination with osteoporosis group (n=28) and the non-combination with osteoporosis group (n=48). The general data of patients was collected, and T scores of lumbar vertebrae and hips of the patients were recorded. **Results:** Duration of diabetes mellitus (DM) and levels of glycosylated hemoglobin A1c (HbA1c), fasting plasma glucose (FPG) and fasting insulin (FINS) level, levels of C-reactive protein (CRP), tumor necrosis factor-alpha (TNF- α) and interleukin 6 (IL-6), level of 24h urine protein (24hndb) of patients in the combination with osteoporosis group was significantly higher, while the value of eGFR was lower than that of patients in the non-combination with osteoporosis group. The bone mineral densities (BMDs) and T scores of lumbar vertebrae and hips of patients in the combination with osteoporosis group were statistically significantly lower than those of patients in the non-combination with osteoporosis group. 24hndb, CRP, TNF- α and IL-6 were all negatively correlated with BMD. Duration of DM, FPG, HbA1c, FINS, 24hndb, BMD and inflammatory cytokines had independent predictive values for patients with diabetic nephropathy combined with osteoporosis. **Conclusion:** 24hndb and inflammatory cytokines are closely related to the combination with osteoporosis in patients with diabetic nephropathy.

Keywords: Diabetic Nephropathy, Osteoporosis, Urine Protein, Inflammatory Cytokine

Introduction

At present, the phenomenon of population aging is getting more and more apparent, and with the constant changing of genetic factors, environment and social lifestyles, the incidence rate of diabetes mellitus (DM) is getting higher and higher in the world¹. According to the latest survey data, the number of patients with DM in China is over 30 million, and most of them are at the age of 45

years old². Besides, the probability of patients with diabetic nephropathy combined with osteoporosis ranges from 10% to 70%³. When chronic kidney disease occurs in DM patients, the abnormal bone mass may occur in patients due to abnormal calcium and phosphorus metabolism, which is manifested as the reduction of bone mass in mild cases and fractures caused by osteoporosis in severe cases⁴. Among patients with chronic kidney disease, the vast majority of them are DM patients, the most common clinical manifestations of which are gradually decreased proteinuria and renal functions⁵. Osteoporosis and DM are chronic metabolic diseases. The mechanism of osteoporosis is the changes in bone tissue structures and the decrease in mineral content in bone⁶, and the long-term indifference leads to the increased fracture rate. Many studies have shown that the urine protein level and the incidence rate of osteoporosis in patients with diabetic nephropathy are significantly higher than those in patients only with DM, which indicates that the severity of impaired renal function

The author has no conflict of interest.

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Edited by: G. Lyrakis
Accepted 19 June 2018



affects the condition of osteoporosis to a large extent⁷. On the other hand, over-expression of inflammatory cytokines plays a vital role in the long-term occurrence and development processes of diabetic nephropathy. The most common inflammatory cytokines include C-reactive protein (CRP), tumor necrosis factor- α (TNF- α) and interleukin 6 (IL-6)⁸. At the same time, inflammatory cytokines also significantly affect the pathophysiology of osteoporosis, so they are closely associated with diabetic nephropathy combined with osteoporosis. Therefore, whether the high incidence rate of osteoporosis is related to the severity of diabetic nephropathy needs to be further studied. The more serious the renal damage is, the higher the incidence rate of osteoporosis will be⁹. With the aggravation of the aging problem, a profound understanding of the risk factors for fractures is essential for early prevention and treatment of osteoporosis.

General data and methods

General data

76 patients diagnosed with diabetic nephropathy in Xintai Affiliated Hospital of Taishan Medical University from May 2016 to December 2016 were selected. Their clinical data were retrospectively analyzed. These patients were divided into the combination with osteoporosis group (n=28) and the non-combination with osteoporosis group (n=48), including 44 males and 32 females aged 55-75 years old with the mean age of 60.46 ± 9.27 years old. All the included patients met the World Health Organization (WHO) diagnostic criteria for DM type 2 in 1997. The diagnostic criteria of diabetic nephropathy were based on the renal function, 24 h urine protein (24hndb) and 24hndb/creatinine ratio, and that of osteoporosis were on the basis of bone mineral density (BMD). Inclusion criteria: patients with complete clinical data who signed the informed consent; patients who were confirmed cases of diabetic nephropathy. Exclusion criteria: Patients with other diabetic complications, such as diabetic retinopathy; patients with the history of osteoporosis; patients with lumbar vertebra and hip diseases who were not suitable for BMD tests; patients suffering from other diseases affecting bone metabolism, such as thyroid disease; patients with parathyroid disease, multiple myeloma or osteopetrosis; patients who perennially took drugs affecting bone metabolism.

Methods

The clinical data of all the patients were retrospectively analyzed, including age, gender, weight, height and the duration of DM, and the body mass index (BMI), blood glucose control and blood glucose treatment methods, including the type, dose and application method of drugs, and the value of eGFR of all the patients were calculated. The formula of eGFR is: $eGFR = 186.3 \times \text{creatinine (SCr)}^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (female) $\text{mL}/(\text{min} \cdot 1.73 \text{ m}^2)$. The stage of renal disease was divided according to EGFR: CKD 1: $eGFR \geq 90 \text{ mL}/\text{min}/1.73 \text{ m}^2$; CKD2: $eGFR = 60 \sim 89 \text{ mL}/\text{min}/1.73 \text{ m}^2$;

CKD3a: $eGFR = 45 \sim 59 \text{ mL}/\text{min}/1.73 \text{ m}^2$; CKD3b: $eGFR = 30 \sim 44 \text{ mL}/\text{min}/1.73 \text{ m}^2$; CKD4: $eGFR = 15 \sim 29 \text{ mL}/\text{min}/1.73 \text{ m}^2$; CKD5: $eGFR < 15 \text{ mL}/\text{min}/1.73 \text{ m}^2$. 6 cases of CKD1, 7 cases of CKD2, 26 cases of CKD3, 31 cases of CKD4 and 6 cases of CKD5 were included in this study.

Fasting peripheral blood samples were taken from all the selected patients after solid and liquid fasting for 10 h overnight. Serum on the upper layer was used for detecting biochemical indicators, including fasting plasma glucose (FPG) detected using the glucose oxidase method, glycosylated hemoglobin A1c (HbA1c) detected using an HbA1c analyzer, fasting insulin (FINS) level measured using the radioimmunoassay with kits provided by Beijing Keep-Science Analysis Sci&Tech Co., Ltd. and other biochemical indicators, such as the total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) determined using the automatic biochemical analyzer provided by Hitachi Ltd. Also, 24 h urine of patients was collected, and 24 h urine sugar (24hnt), 24hndb and 24 h microalbuminuria (24hnlwdb) were measured using the UniCel Dx C 800 Synchron fully automatic biochemical analyzer. 10 mL peripheral blood was taken from patients after solid and liquid fasting for 10 h overnight, and serum on the upper layer was used for detecting inflammatory cytokines, CRP, TNF- α , and IL-6, using the immune turbidimetric assay. Reagents and instruments were provided by Shandong Bio-Instrumentation Co. Ltd.

Determinations of BMD: Lumbar vertebrae and hips were selected for BMD measurement. Patients were placed in the supine position, and their arms were crossed in the chest away from lumbar vertebrae and hips; No. 1-4 lumbar vertebrae were selected to determine the front and rear BMDs, and the determination in the hips led to hip adduction and internal rotation of patients; the Wards triangle, greater trochanter and femoral shaft were measured; all the determinations of BMD were achieved using the BMD automatic analyzer provided by LG Electronics, Germany, and T score was calculated; T score of BMD (BMDT) less than -2.5 standard deviation (SD) represented osteoporosis.

Statistical methods

Data were processed using the Statistical Product and Service Solutions (SPSS) 19.0 software, and the collected data were expressed as mean \pm standard deviation (SD). χ^2 test was used to compare count data, the correlation analysis was used to analyze two factors, and the logistic analysis was used to analyze relevant risk factors. $p < 0.05$ represented that the difference was statistically significant.

Results

Comparisons of general data of patients with diabetic nephropathy between the combination with osteoporosis group and the non-combination with osteoporosis group

There were no statistical differences in age, gender, BMI and levels of TC, TG, LDL and HDL between the combination with osteoporosis group and the non-combination with

Table I. Comparisons of general data of patients with diabetic nephropathy between the combination with osteoporosis group and the non-combination with osteoporosis group.

General data	Combination with osteoporosis group (n=28)	Non-combination with osteoporosis group (n=48)	p value
Age (years old)	61.59±9.5	60.72±9.8	0.721
Gender (male/female)	16/12	28/20	0.153
Duration of DM (year)	11.56±2.04	6.85±3.91	0.001
BMI (kg/m ²)	25.73±2.59	23.97±3.30	0.565
HbA1c (%)	10.58±1.39	7.36±0.86	0.000
FPG (mmol/L)	11.42±1.88	8.39±0.97	0.001
FINS (mIU/L)	14.26±2.38	9.86±2.42	0.003
TC (mmol/L)	5.76±1.02	4.94±1.03	0.068
TG (mmol/L)	1.94±1.05	1.92±0.97	0.072
LDL (mmol/L)	3.08±0.98	2.98±0.75	0.588
HDL (mmol/L)	1.47±0.49	1.42±0.33	0.082

Table II. Comparisons of levels of inflammatory cytokines in patients with diabetic nephropathy between the combination with osteoporosis group and the non-combination with osteoporosis group.

Relevant indicator	Combination with osteoporosis group (n=28)	Non-combination with osteoporosis group (n=48)	p value
CPR (mg/L)	5.45±1.44	2.36±0.98	0.001
TNF- α (ng/mL)	11.32±1.18	6.49±1.08	0.001
IL-6 (μ g/L)	9.23±1.66	7.28±1.19	0.000

Table III. Comparisons of urine proteins in patients with diabetic nephropathy between the combination with osteoporosis group and the non-combination with osteoporosis group.

Urine protein	Combination with osteoporosis group (n=28)	Non-combination with osteoporosis group (n=48)	p value
24hnt (mmol/h)	59.4±75.3	51.7±74.9	0.579
24hndb (g/24h)	0.16±0.39	0.53±1.41	0.031
24hnlbdb (mg/24h)	85.76±331.95	70.07±188.21	0.496

Table IV. Comparison of BMD of patients with diabetic nephropathy between the combination with osteoporosis group and the non-combination with osteoporosis group.

Group	Lumbar vertebra (g/cm ²)	Hip (g/cm ²)	T score of lumbar vertebrae	T score of hips
Non-combination with osteoporosis group	1.531±0.129	1.041±0.103	0.7±1.1	0.4±0.8
Combination with osteoporosis group	0.795±0.093	0.592±0.135	-2.4±0.7	-2.1±0.8
p value	0.026	0.001	0.001	0.001

osteoporosis group ($p>0.05$), but duration of DM in combination with osteoporosis group was longer than that in the non-combination with osteoporosis group, and levels of HbA1c, FPG, and FINS in the former were significantly higher than those in the latter (Table I).

Comparisons of levels of inflammatory cytokines in patients with diabetic nephropathy between the combination with osteoporosis group and the non-combination with osteoporosis group

Levels of CRP, TNF- α , and IL-6 in combination with osteoporosis group were significantly higher than those in the non-combination with osteoporosis group, and the differences were statistically significant ($p<0.05$) (Table II).

Comparisons of urine proteins in patients with diabetic nephropathy between the combination with osteoporosis group and the non-combination with osteoporosis group

There were no statistical differences in levels of 24hnt and 24hnlbdb between the two groups of included patients ($p>0.05$), but the level of 24hndb of patients in combination with osteoporosis group was significantly higher, and the level of eGFR was considerably lower than that of patients in the non-combination with osteoporosis group ($p<0.05$) (Table III).

Comparison of BMD of patients with diabetic nephropathy between the combination with osteoporosis group and the non-combination with osteoporosis group

Comparison of the condition of osteoporosis between the two groups of patients showed that BMDs and T scores of lumbar vertebrae and hips of patients in combination with osteoporosis group were significantly lower than those in the non-combination with osteoporosis group, and the differences were statistically significant ($p<0.05$) (Table IV).

Correlation analyses of 24hndb and inflammatory cytokines (CRP, TNF- α , and IL-6) with BMD

24hndb ($r=-0.722$, $p<0.001$), CRP ($r=-0.661$, $p<0.001$), TNF- α ($r=-0.537$, $p<0.001$) and IL-6 ($r=-0.793$, $p<0.001$) were negatively correlated with BMD of the included patients with diabetic nephropathy in the combination with osteoporosis group and the non-combination with osteoporosis group (Figure 1-4).

Logistic regression analysis of risk factor prediction for patients with diabetic nephropathy combined with osteoporosis

Duration of DM, FPG, HbA1c, FINS, 24hndb, BMD and inflammatory cytokines (CRP, TNF- α , and IL-6) had independent predictive values for patients with diabetic nephropathy combined with osteoporosis, and the differences were statistically significant ($p<0.05$) (Table V).

Discussion

With the continuous changes in social lifestyles, including the living environment and eating habits, the incidence rate of DM is increased year by year. In particular, the incidence rate of DM in China reaches close to 10%, the vast majority of which belong to DM type 2 cases¹⁰. Dysfunction of blood glucose regulation results in impaired functions of various target organs in the whole body. The most common clinical symptom is vascular disease¹¹, and diabetic nephropathy is one of the common complications. Diabetic nephropathy can easily lead to osteoporosis, and the impaired islet function of DM triggers the absolute or relative deficiency in insulin secretion, thus resulting in the chronic metabolic disorders in body glucose or other indicators¹². This will lead to the imbalance of calcium and phosphorus metabolism, which eventually results in bone metabolism, manifested as osteoporosis or even fractures, increasing the mortality rate¹³. In patients with diabetic nephropathy, vitamin D synthesis is significantly insufficient, and the lack of vitamin D may aggravate the degree of insulin resistance¹⁴, improve the synthesis and secretion of urine microalbumin and decrease the activity of renal hydroxylase. The decreased content of 1,25 dihydroxy vitamin D₃ [1,25-(OH)₂D₃]¹⁵ reduces the absorption of calcium through the small intestine mucosa and bone calcium formation, so the severity of diabetic nephropathy affects bone metabolism¹⁶. Parathyroid cells and parathormone (PTHs) are also affected by 1, 25-(OH)₂D₃ as the decreased level of 1, 25-(OH)₂D₃ reduces PTH inhibition, increases *in vivo* levels, triggers secondary hyperparathyroidism¹⁷ and improves bone resorption, thus aggregating the condition of osteoporosis to a large extent¹⁸. If patients stay under the condition of chronic kidney disease for a long time, electrolyte metabolism disorders, chronic metabolic acidosis, increased bone mineral soluble content and reduced bone mineralization will occur, and a large number of data have shown that hypophosphatemia can cause osteomalacia to a large extent¹⁹. In addition, chronic inflammation plays an essential role in the processes of diabetic nephropathy and insulin resistance, and the level of inflammatory cytokines *in vivo* affects the degree of insulin resistance²⁰.

It was found in this study that levels of CRP, TNF- α , IL-6 and 24hndb in patients with diabetic nephropathy in combination with osteoporosis group were higher than those in the non-combination with osteoporosis group; BMDs and T scores of lumbar vertebrae and hips in the former were significantly lower than those in the latter, and the differences were statistically significant ($p<0.05$); 24hndb and inflammatory cytokines (CRP, TNF- α , and IL-6) were negatively related to BMD, and 24hndb, BMD and inflammatory cytokines (CRP, TNF- α , and IL-6) had independent predictive values for patients with diabetic nephropathy combined with osteoporosis. At the same time, a large number of studies have shown that with the decline of renal cell function, urine protein is gradually increased in diabetic patients, which induces metabolic abnormalities of

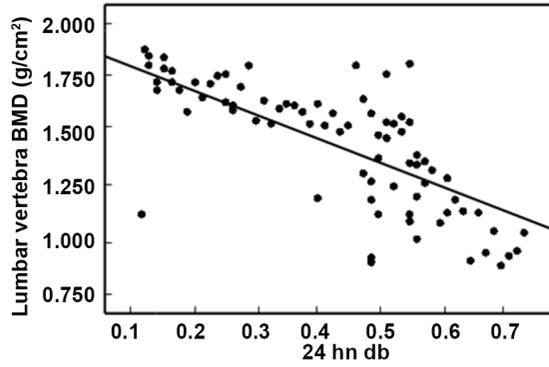


Figure 1. Correlation between 24hndb and lumbar vertebra BMD.

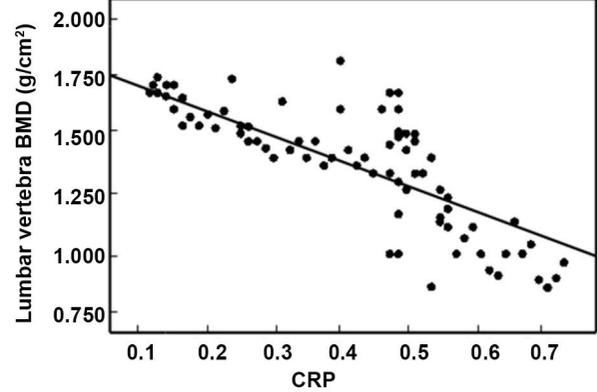


Figure 2. Correlation between CRP and lumbar vertebra BMD.

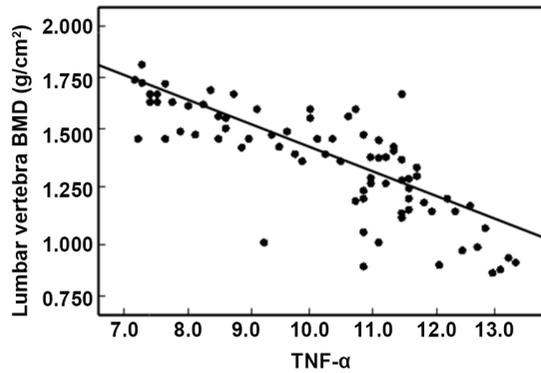


Figure 3. Correlation between TNF-α and lumbar vertebra BMD.

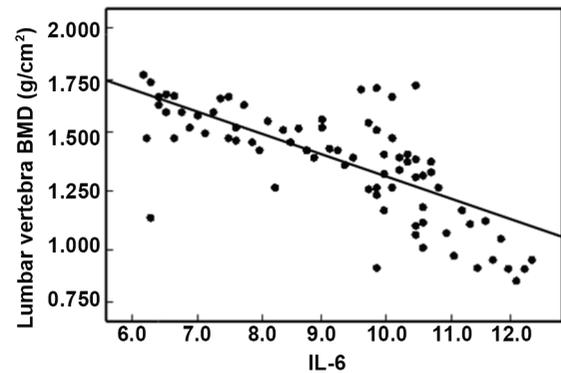


Figure 4. Correlation between IL-6 and lumbar vertebra BMD.

Table V. Logistic regression analysis of risk factor prediction for patients with diabetic nephropathy combined with osteoporosis.

Factor	p value	Odds ratio (OR) value	95% confidence interval (95% CI)
Age	0.109	1.095	0.956, 1.148
Gender	0.779	0.851	0.992, 5.979
Duration of DM	0.013	7.543	1.918, 20.147
BMI	0.851	0.068	0.443, 20.305
FPG	0.036	1.017	0.954, 1.019
HbA1c	0.032	1.006	0.929, 1.113
FINS	0.019	1.045	0.997, 1.314
CRP	0.028	1.053	0.985, 1.064
TNF-α	0.005	7.348	1.918, 22.149
IL-6	0.026	1.029	0.974, 1.081
24hndb	0.048	1.335	0.579, 4.016
Lumbar vertebra BMD	0.017	8.053	1.814, 9.525
Hip BMD	0.035	1.274	0.623, 3.928
TC	0.024	1.396	0.576, 3.572
TG	0.526	1.447	0.465, 4.491
LDL	0.961	0.082	0.445, 20.305
HDL	0.322	0.989	0.991, 5.035

many cytokines in the body, such as Hcyl, blood lipid level and so on. The reason was that these inflammatory cytokines enhanced bone and skeleton resorption and the formation of osteoclasts, resulting in excessive production of macrophages interleukin 1, and low-level inflammatory cytokines could protect islet β cells to a certain degree¹⁸. There are also many limitations to this study, such as the small number of patients included, and the insufficient study of cytokines that affect the stages of renal disease. These defects will be noted in the relevant studies in the future. The content of urine proteins, the severity of chronic kidney disease and the level of inflammatory cytokines in patients with diabetic nephropathy are risk factors for osteoporosis. Patients with diabetic nephropathy need to have a profound understanding of the risk factors influencing BMD for early prevention or treatment of osteoporosis.

Ethics approval and consent to participate

The study was approved by the ethics committee of Xintai Affiliated Hospital of Taishan Medical University and informed consents were signed by the patients and/or guardians.

Consent for publication

Informed consents were signed by the patients and/or guardians.

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