Research Paper

Correlation Study of Serum Dickkopf 4 Level with Metabolic Syndrome and Carotid Atherosclerosis in Patients with Type 2 Diabetes Mellitus

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To investigate the characteristics of Dickkopf 4 changes in patients with type 2 diabetes mellitus and its correlation with carotid atherosclerosis, is the objective of the study. A cross-sectional survey was performed and eligible patients with type 2 diabetes mellitus were taken as the subjects. Lifestyle, physical measurements, blood biochemical parameters and relevant imaging examinations were collected from all participating subjects. 237 type 2 diabetes mellitus patients were included in present study and divided into four groups according to the quartiles of serum Dickkopf 4. The incidence of body mass index, fasting plasma glucose, fasting C-peptide, glycated haemoglobin, insulin resistance index, total cholesterol, triglycerides, low-density lipoprotein, C-reactive protein, high-density lipoprotein, and non-alcoholic fatty liver disease, carotid atherosclerosis were compared between groups, p<0.05. Dickkopf 4 was positively correlated with body mass index, C-reactive protein, fasting plasma glucose, fasting C-peptide, glycated haemoglobin, total cholesterol, triglycerides, low-density lipoprotein, insulin resistance index and creatinine, and negatively correlated with high-density lipoprotein and estimated glomerular filtration rate, p<0.05. Non-metabolic syndrome group and metabolic syndrome group were compared and non-carotid atherosclerosis group and carotid atherosclerosis group were compared for all the parameters (p<0.05). High Dickkopf 4 was an independent risk factor for metabolic syndrome (odds ratio=2.023, 95 % confidence interval: 1.042-3.926, p=0.037). Binary logistic regression analysis, high Dickkopf 4 was an independent risk factor for carotid atherosclerosis (odds ratio=2.513, 95 % confidence interval: 1.379-2.513, p=0.003). High Dickkopf 4 is a risk factor for hypertension and non-alcoholic fatty liver disease in type 2 diabetic patients and an independent risk factor for metabolic syndrome and carotid atherosclerosis in type 2 diabetic mellitus patients.

Key words: Diabetes mellitus, metabolic syndrome, Dickkopf 4, carotid atherosclerosis

As people's economic power rises, their living standards improve and their lifestyles change, more and more unreasonable diets high in calories, fat and low in fiber are being consumed, while physical work and exercise are increasingly lacking, and people are consuming far more energy than they consume, which promotes the development of more and more metabolic diseases[1]. In 1980s, the prevalence of diabetes in China was only 0.609 %[2]. According to the survey data of the International Diabetes Federation, the number of diabetes patients in the world has reached 366 million at present and is expected to increase to 552 million by 2030, with Type 2 Diabetes Mellitus (T2DM) accounting for 85 % to 95 % of the total population with diabetes, and the incidence of diabetes continues to rise worldwide[3]. The high incidence and disability of diabetes and its life-threatening complications pose a great threat to human health, put enormous economic pressure on families and impose a major burden on society.

T2DM is a metabolic disease of the body and is a type of diabetes, and diabetic macrovascular disease is the main complication of T2DM, and also an important reason of death and disability in T2DM patients. Carotid atherosclerosis and atheromatous plaque formation are the main pathological basis of macroangiopathy and early detection and intervention of atherosclerosis is of great importance for the comprehensive treatment

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of T2DM. The pathogenesis of atherosclerosis is not fully understood, but, many studies have confirmed that inflammation is involved in the whole process of atherosclerosis from the formation of lipids to the eventual rupture of plaques[4]. Studies have shown that inflammatory cells migrate subcutaneously and differentiate into macrophages, which absorb lipids and become foam cells. Foam cells can release various inflammatory mediators and promote the generation and development of atherosclerotic plaques[5]. Interleukin-6 (IL-6) can cause cell necrosis and promote endothelial damage, which further induces the expression of platelet-derived growth factor, Tumor Necrosis Factor (TNF), macrophage colony-stimulating factor, etc., and promotes smooth muscle cell proliferation, and insulin resistance and persistent hyperglycemia in type 2 diabetic patients are also involved in this inflammatory process. Oxidative stress is also involved in the formation and development of arterial plaques and oxidative modification of Low-Density Lipoprotein (LDL) and oxidative stress in vascular endothelial cells can contribute to the formation of vulnerable plaques. Other factors such as the nature of the plaque and the external forces on the plaque can contribute to the development, progression and even rupture of the plaque[6].

The Dickkopf (DKK) family is a group of secreted glycoproteins that in vertebrates consists of four members, named DKK1-4, ranging in size from 255 to 350 amino acids. The DKK4 gene (also known as the Dickkopf homolog 4 gene) is located in the 8p11.2-p11.1 region of chromosome 8. DKK4 acts as a repressor of Wingless-related integration site (Wnt) proteins and is involved in the transduction of the Wnt signaling pathway, which in turn regulates cell differentiation and apoptosis[7]. The results showed that the damaged carotid arteries showed significant macrophage accumulation and intimal hyperplasia, while the experimental group was given artificial ligands of DKK4, and the treated mice showed significant hyperlipidemia-induced vascular injury and plaque formation. This suggests that DKK4 may be correlated with atherosclerosis, but we have not found any report on the correlation between DKK4 and carotid atherosclerosis in T2DM.

The objective of this study is to analyze the characteristics of serum DKK4 in type 2 diabetic patients and its relationship with atherosclerotic plaques, to provide a theoretical basis for the elucidation of the role of DKK4 in the development of carotid atherosclerosis in type 2 diabetic patients, and to explore the value of DKK4 in the early diagnosis and intervention of diabetic carotid atherosclerosis and other macrovascular complications.

MATERIALS AND METHODS

Subjects:

Inclusion criteria: Patients with T2DM aged 18 y or older who were seen in the endocrinology department of our hospital between January 2019 and December 2020, all of whom had a stable metabolic status (no diabetes-related acute complications such as infections, hypoglycemia, ketoacidosis, hyperosmolar hyperglycemic coma and other stressful events).

Exclusion criteria: Presence of any disease that may affect metabolic status such as hyper or hypothyroidism, nephrotic syndrome, acute and chronic renal insufficiency requiring or undergoing renal replacement therapy, patients with viral hepatitis or other liver disease, gout and cerebrovascular accidents; pregnant and lactating women; patients who are using related drugs such as febuxostat, benzbromarone, allopurinol, cloxacillin and tachyphylaxis tablets that can affect serum DKK4 levels; patients without complete clinical information. Clinical information and medical history were obtained through medical record review, including age, sex, duration of diabetes, drinking and smoking habits, family history and the use of glucose-lowering drugs, Cardiovascular and Cerebrovascular Events (CCEs), lipid-lowering drugs and anti-hypertensive medications were provided.

Physical examination:

The weight (kg), height (m) and blood pressure (mmHg) of subjects were evaluated. For measurement of height and weight, the subject wore a single garment, removed shoes and hat, fasted and emptied the bladder, stood upright on the height and weight meter and looked straight ahead. Body Mass Index (BMI) was calculated by dividing the formula weight by the square of height (kg/m^2). Blood pressure was measured by a calibrated mercury column standard cuff sphygmomanometer with a resting position for at least 10 min and the right upper arm blood pressure was measured twice and averaged (more than 2 min apart).

Laboratory tests:

Venous blood was taken from all subjects after fasting for at least 10 h. Laboratory measurements included glycated Haemoglobin (HbA1c), Fasting Plasma Glucose (FPG), fasting Insulin (INS), Fasting C-Peptide (FCP), Total Triglycerides (TGs), Total Cholesterol (TC), Low-Density Lipoprotein Cholesterol (LDL-C), High-Density Lipoprotein Cholesterol (HDL-C), Creatinine (Cr), C-Reactive Protein (CRP), DKK4, Serum Uric Acid (SUA) and 24 h Urinary Albumin Excretion (UAE).

A simplified Modification of Diet in Renal Disease (MDRD) formula was used to estimate the glomerular filtration rate.

The estimated Glomerular Filtration Rate (eGFR) (ml/min)=186×{[Serum Cr (μmol/l)/88.4]$^{-1.154}$}×Age$^{-0.203}$×0.742 (Female).

The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) (mIU-mmol/l)=INS (mIU/l)×fasting glucose (mmol/l)/22.5 HOMA-IR (C-Peptide)=1.5+fasting glucose×FCP/2800 (F=5.511, p=0.029).

Imaging:

Carotid Doppler ultrasonography includes measurements of carotid atherosclerotic plaque and lumen stenosis. The intima-media thickness of carotid artery is the distance between the interface of vascular lumen-intima and media-adventitia.

Diagnostic criteria:

Hyperuricaemia: Fasting serum DKK4 levels were measured twice on non-same day and diagnostic criteria was at least 420 μmol/l in men, 360 μmol/l in women and 420 μmol/l in postmenopausal women on a normal purine diet.

Hypertension: A Systolic Blood Pressure (SBP) of at least 140 mmHg or a diastolic blood pressure of at least 90 mmHg measured at the clinic in a calm state, or a definite diagnosis of hypertensive disease treated with antihypertensive medication.

The diagnosis of diabetes mellitus is based on the 1999 World Health Organization (WHO) diagnostic criteria for diabetes mellitus. Those with typical symptoms of three or more, plus fasting blood glucose≥7.0 mmol/l or random blood glucose over 11.1 mmol/l or 2 h oral glucose tolerance test over 11.1 mmol/l; those without typical symptoms with atleast 2 and non-same day blood glucose measurements meeting these criteria.

The diagnosis of metabolic syndrome is based on the 2005 International Diabetes Federation Global Consensus on Metabolic Syndrome and requires at least three of the following:

Abdominal obesity-Waist circumference>90 cm for men and >85 cm for women; TG level≥1.70 mmol/l (150 mg/dl) or have received specific treatment for this lipid abnormality; HDL-C levels<1.03 mmol/l (40 mg/dl) in men and <1.30 mmol/l (50 mg/dl) in women; SBP≥130 mmHg or diastolic blood pressure≥85 mmHg or previously diagnosed hypertension under treatment; fasting blood glucose level≥5.6 mmol/l (100 mg/dl) or history of diabetes mellitus.

According to WHO standards, a BMI≥18.5 kg/m^2 but<24.9 kg/m^2 is considered normal, less than 18.5 kg/m^2 is defined as thin, a BMI≥25 kg/m^2 but <29.9 kg/m^2 is considered overweight and a BMI≥30 kg/m^2 is considered obese.

Carotid atherosclerosis: Application of color Doppler ultrasound and flow imaging to visualize the intima, including the presence of carotid atherosclerotic plaques and measure their size, whether the lumen is narrowed. Stenosis of the carotid arteries (common carotid artery, carotid balloon, carotid artery) was defined as >50 % reduction in the luminal internal diameter of the vessel. The presence of plaque was defined as ≥1 in any carotid plaque.

Daily consumption of alcohol converted to ethanol>20 g (>140 g/w) for men and >10 g (>70 g/w) for women is considered as regular alcohol consumption. Smoking was defined as smoking more than 1 cigarette per day for a continuous or cumulative period of more than 6 mo.

Referring to the 2010 guidelines for the diagnosis of NAFLD, the following 3 criteria should be met for the diagnosis of Non-Alcoholic Fatty Liver Disease (NAFLD).

No history of alcohol consumption and consumption of less than 140 g/w of ethanol (less than 70 g/w for women); specific diseases capable of developing fatty liver, such as drug-related liver disease, total parenteral nutrition, viral hepatitis, autoimmune liver disease and hepatomegaly; histological changes on liver biopsy meet the pathological diagnostic criteria for fatty liver disease.

Imaging of the liver consistent with diffuse fatty liver with no other explainable cause is the working definition.

Liver biopsy is an invasive procedure that requires a specialist to complete and obtain histopathology and since it is not easy to actually complete this paper on diagnostic criteria #3, its working definition was used for this diagnostic condition in this study.

The diagnosis of coronary atherosclerotic heart disease is based on the 2010 guidelines "Diagnostic criteria for coronary atherosclerotic heart disease (WS 319-2010)".

Statistical analysis:

The study data were analyzed using Statistical Package for the Social Sciences (SPSS) 19 software and divided into four groups based on the DKK4 quartiles of the study population and for normally distributed continuous variables, one-way Analysis of Variance (ANOVAs) and Least Significant Difference (LSD) were used for comparison and the results were expressed as mean±standard deviation. For skewed distribution data, Mann-Whitney U test was used and the results were expressed as median and quartiles. Categorical variables were expressed as frequencies and percentages. The t-test and Pearson's chi-square test were used to analyze the differences between groups for normally distributed variables and categorical variables, respectively. The strength of correlation between continuous and categorical variables and the target variable was statistically analyzed using Pearson analysis and Spearman analysis, respectively. Multifactor analysis was performed using binary logistic regression models, $p<0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Basic anthropological data, serological indicators of the study population was shown here. A total of 237 T2DM patients were included in this study, 167 (70.46 %) were males and 70 (29.54 %) were females and the mean age of all the patients were 51.45±12.54. The number of hyperuricemia was 81 (34.18 %) and 156 (65.82 %) had normal serum DKK4 levels (Clinical characteristics and blood biochemical parameters of the study population are specified in Table 1). There were four groups divided according to quartiles of serum DKK4 and there were statistically significant differences between the groups in the incidence of BMI, fasting glucose, FCP, HbA1c, insulin resistance index, TC, TGs, LDL, CRP, HDL, NAFLD and carotid atherosclerosis ($p<0.05$).

TABLE 1: CLINICAL CHARACTERISTICS AND BLOOD BIOCHEMICAL PARAMETERS OF PATIENTS WITH T2DM

Variables	Q1 (n=60) ≤259 µmol/l	Q2 (n=59) 260-326 µmol/l	Q3 (n=59) 327-394 µmol/l	Q4 (n=59) >395 µmol/l	p value
DKK4 (µmol/l)	224 (183-240)	287 (270-309)	363 (342-374)	450 (419-491)	<0.001
Age (years)	52.6±12.8	50.8±11.4	51.0±12.7	51.5±13.5	0.866
Male (n, %)	33 (55 %)	45 (76.3 %)	40 (67.8 %)	49 (83.1 %)	0.006
Duration of Diabetes (DD) (months)	80.7±60.2	83.9±71.5	78.0±74.9	72.1±70.0	0.82
Smoking (n, %)	19 (31.7 %)	29 (49.2 %)	29 (49.2 %)	30 (50.8 %)	0.114
Alcohol (n, %)	11 (18.3 %)	19 (32.2 %)	24 (40.7 %)	22 (37.3 %)	0.047
Hypertension (n, %)	17 (28.3 %)	25 (42.4 %)	23 (39.0 %)	28 (47.5 %)	0.179
NAFLD (n, %)	23 (38.3 %)	36 (61.0 %)	34 (57.6 %)	47 (79.7 %)	<0.001
Carotid plaque (n, %)	27 (45.0 %)	22 (37.3 %)	24 (40.7 %)	38 (64.4 %)	0.015
Coronary Artery Disease (CAD) (n, %)	1 (1.7 %)	4 (6.8 %)	1 (1.7 %)	5 (8.5 %)	0.177
BMI (kg/m^2)	22.31±2.89	25.60±3.84	24.74±3.62	25.76±3.10	<0.001
FPG (mmol/l)	6.89±0.98	7.22±1.04	7.57±1.04	8.23±1.15	<0.001

C-peptide	1.28±1.15	1.52±0.78	1.75±0.99	2.27±1.28	<0.001
Insulin	95.69±136.06	77.90±86.20	74.63±56.94	80.30±47.58	0.579
HbA1C (%)	8.2 (7.2-9.1)	8.7 (7.9-93)	8.6 (7.4-9.6)	83 (7.2-8.9)	0.028
HOMA-IR	4.04 (3.09-5.18)	5.31 (3.84-7.07)	5.96 (4.30-7.55)	7.59 (5.58-9.86)	<0.001
TC (mmol/l)	4.30±0.91	4.36±0.88	4.82±0.98	4.85±0.99	0.001
TG (mmol/l)	0.93 (0.62-1.29)	1.50 (1.05-1.72)	1.64 (1.37-1.79)	1.87 (1.35-2.13)	<0.001
LDL-C (mmol/l)	2.16±0.54	2.66±0.61	2.71±0.57	3.12±0.61	<0.001
HDL-C (mmol/l)	1.18±0.29	1.06±0.22	1.10±0.31	1.02±0.28	0.012
Cr (μmol/l)	77.56±57.53	85.01±62.15	78.51±13.29	91.03±38.24	0.368
eGFR (ml/min)	99.70±16.82	97.62±17.11	96.60±16.41	90.10±20.79	0.025
UAE (mg/24 h)	187.62±1116.24	166.71±444.27	82.17±232.28	301.00±940.76	0.499
CRP (mg/l)	4.94±1.40	5.08±1.21	5.27±1.53	5.93±2.26	0.007

Correlation analysis of serum DKK4 with anthropological data and serological indicators was shown in Table 2. Based on the study observation serum DKK4 was correlated with related metabolic and clinical morbidity as follows. Pearson correlation analysis showed that serum DKK4 levels were positively correlated with BMI, CRP, fasting glucose, FCP, HbA1c, TC, TGs, LDL, insulin resistance index, Cr and positively correlated with HDL, eGFR and all were statistically different ($p<0.05$). According to Spearman correlation analysis, high DKK4 was strongly associated with sex/male, smoking, alcohol consumption, hypertension, fatty liver and carotid atherosclerosis compared to normal DKK4 levels, all were statistically significant ($p<0.05$).

TABLE 2: CORRELATION STUDY BETWEEN DKK4 AND OTHER INDICATORS IN T2DM PATIENTS

Variables	Correlation coefficient	p value
Age	-0.010	0.882
Male	0.211	0.001
DD	-0.020	0.755
Smoking	0.146	0.024
Alcohol	0.164	0.011
Metabolic syndrome	0.317	<0.001
Hypertension	0.158	0.015
NAFLD	0.300	<0.001
CAD	0.096	0.140
BMI	0.288	<0.001
Glucose	0.477	<0.001
C-peptide	0.401	<0.001
Insulin	-0.018	0.786
HbA1C	0.288	<0.001
HOMA-β	0.043	0.508
HOMA-IR	0.477	<0.001
TC	0.252	<0.001
TGs	0.599	<0.001
LDL-C	0.445	<0.001
HDL-C	-0.155	0.017
Cr	0.143	0.027
eGFR	-0.268	<0.001
UAE	0.115	0.077
CRP	0.284	0.001
Carotid atherosclerosis	0.161	0.013

Comparison of anthropological data, serological indicators and clinical characteristics and other relevant parameters between the two groups in all diabetic patients grouped by the presence or absence of metabolic syndrome is shown here. From Table 3 it is clear that serum DKK4 is associated with several metabolic indices and metabolic syndrome is a syndrome of abnormal accumulation of metabolic components in the body, further the study population was divided into metabolic syndrome group (n=141) as well as non-metabolic syndrome group (n=96). Compared to non-metabolic syndrome group, patients with metabolic syndrome had higher serum DKK4, BMI, fasting glucose, FCP, insulin resistance index, TGs, UAE and higher prevalence of hypertension and fatty liver with low HDL levels, all of which were statistically significant ($p<0.05$).

TABLE 3: CLINICAL CHARACTERISTICS AND BLOOD BIOCHEMICAL INDICES OF PATIENTS IN THE METABOLIC SYNDROME AND NON-METABOLIC SYNDROME GROUPS

Variables	Metabolic syndrome (n=141)	Non-metabolic syndrome (n=96)	p value
DKK4 (μmol/l)	359.05±101.51	292.73±89.90	<0.001
Age (years)	51.03±12.61	52.07±12.74	0.530
Male (n, %)	103 (73.0 %)	64 (66.7 %)	0.312
DD (months)	72 (24-120)	66 (12-120)	0.803
Smoking (n, %)	65 (46.1 %)	42 (43.8 %)	0.791
Alcohol (n, %)	50 (35.5 %)	26 (27.1 %)	0.203
Hypertension (n, %)	72 (51.1 %)	21 (21.9 %)	<0.001
NAFLD (n, %)	104 (73.8 %)	36 (37.5 %)	<0.001
CAD (n, %)	8 (5.7 %)	3 (3.1 %)	0.279
BMI (kg/m^2)	26.01±3.55	22.51±2.63	<0.001
FPG (mmol/l)	7.62±1.15	7.27±1.14	0.023
C-peptide	2.03±1.13	1.22±0.93	<0.001
Insulin	68.54 (50.01-97.58)	56.45 (36.28-80.10)	0.579
HOMA-β	3.28 (2.23-4.97)	2.52 (1.68-3.74)	0.001
HOMA-IR	6.33 (4.93-8.14)	4.10 (3.02-5.88)	<0.001
TC (mmol/l)	4.60±0.95	4.56±0.99	0.717
TTG (mmol/l)	1.63±0.48	1.18±0.45	<0.001
LDL-C (mmol/l)	2.74±0.63	2.57±0.73	0.087
HDL-C (mmol/l)	0.99±0.25	1.23±0.27	<0.001
Cr (μmol/l)	78.00 (70.50-86.25)	75.95 (68.43-82.50)	0.077
eGFR (ml/min)	97.58 (90.77-108.88)	99.12 (91.83-107.08)	0.689
UAE (mg/24 h)	20.34 (11.12-60.30)	9.77 (6.28-22.45)	0.001
CRP (mg/l)	5.24 (4.26-6.06)	5.14 (4.39-5.74)	0.448

Comparison of anthropological data, serological indicators and clinical characteristics and other relevant parameters between the two groups in the study population grouped by the presence or absence of carotid atherosclerosis was shown in Table 4. In this study, to clarify the relationship between serum DKK4 levels and carotid atherosclerosis, the study population was divided into carotid atherosclerosis group (n=111) and non-carotid atherosclerosis group (n=126) on the basis of Table 4. Compared to the non-carotid atherosclerosis group, patients in the carotid atherosclerosis group had higher prevalence of serum DKK4, age, duration of diabetes, BMI, fasting glucose, FCP, insulin resistance index, TGs, UAE, Cr and a higher prevalence of hypertension and a lower glomerular filtration rate, all with statistically significant differences ($p<0.05$).

Binary logistic analysis of serum DKK4, metabolic syndrome and carotid atherosclerosis was shown in Table 5. In this study, to clarify the relationship between serum urea and metabolic syndrome and carotid atherosclerosis, DKK4, hypertension, fatty liver, 24 h UAE and fasting glucose were included in the binary logistic regression model for metabolic syndrome and DKK4, hypertension, duration of diabetes mellitus, BMI and smoking were included in the binary logistic regression model for carotid atherosclerosis based on the results obtained in Table 3 and Table 4. We found that patients with serum DKK4>420 μmol/l in men and >360 μmol/l in women (<420 μmol/l in postmenopausal women) developed metabolic syndrome and carotid atherosclerosis compared with patients with serum DKK4<420 μmol/l in men and <360 μmol/l in women (>420 μmol/l in postmenopausal women). The risk of metabolic syndrome and carotid atherosclerosis was higher in patients with serum DKK4>420 μmol/l in men and >360 μmol/l in women (>420 μmol/l in postmenopausal women), with a dominance ratio of 2.023 and 2.513, respectively ($p<0.05$, Table 5 and Table 6).

TABLE 4: CLINICAL CHARACTERISTICS AND BLOOD BIOCHEMICAL PARAMETERS OF PATIENTS IN THE CAROTID ATHEROSCLEROSIS AND NON-CAROTID ATHEROSCLEROSIS GROUPS

Variables	Carotid atherosclerosis (n=111)	Non-carotid atherosclerosis (n=126)	p value
DKK4 (μmol/l)	352.17±112.62	314.58±88.69	0.004
Age (years)	55.19±11.90	48.16±12.20	<0.001
Male (n, %)	79 (71.2 %)	88 (69.8 %)	0.887
DD (months)	84 (48-120)	48 (12-111)	0.002
Smoking (n, %)	50 (45.0 %)	57 (45.2 %)	1.000
Alcohol (n, %)	33 (29.7 %)	43 (34.1 %)	0.489
Hypertension (n, %)	56 (50.5 %)	37 (29.4 %)	0.001
NAFLD (n, %)	71 (64.0 %)	69 (54.8 %)	0.186
CAD (n, %)	7 (6.3 %)	4 (3.2 %)	0.202
BMI (kg/m^2)	24.87±3.78	24.35±3.50	0.269
FPG (mmol/l)	7.73±1.18	7.26±1.10	0.002
C-peptide	1.87±1.28	1.56±0.95	0.035
Insulin	69.20 (50.08-100.40)	57.35 (37.35-83.82)	0.579
HbA1C (%)	8.43±1.42	8.29±1.45	0.460
HOMA-β	3.35 (2.30-5.06)	2.60 (1.71-3.77)	<0.001
HOMA-IR	6.18 (4.23-7.86)	5.18 (3.69-7.15)	0.016
TC (mmol/l)	4.58±1.03	4.58±0.92	0.991
TTG (mmol/l)	1.53±0.53	1.38±0.50	0.032
LDL-C (mmol/l)	2.69±0.76	2.64±0.60	0.601
HDL-C (mmol/l)	1.06±0.31	1.11±0.28	0.200
Cr (μmol/l)	79.00 (70.50-90.70)	76.00 (69.30-82.30)	0.035
eGFR (ml/min)	96.02 (82.47-106.27)	101.96 (93.03-109.97)	0.002
UAE (mg/24 h)	32.77 (15.22-148.07)	9.77 (6.28-22.45)	<0.001
CRP (mg/l)	5.30 (4.29-6.08)	5.11 (4.38-5.81)	0.089

TABLE 5: CORRELATION ANALYSIS OF SERUM DKK4 AND METABOLIC SYNDROME

Characteristics	Odds ratio	95 % Confidence Interval (CI)	p value
DKK4	2.023	1.042-3.926	0.037
Hypertension	3.164	1.655-6.047	<0.001
NAFLD	3.962	2.185-7.184	<0.001
FPG (>7 mmol/l)	0.844	0.450-1.581	0.595
UAE (>30 mg)	1.287	0.662-2.501	0.456

TABLE 6: CORRELATION ANALYSIS OF SERUM DKK4 AND CAROTID ATHEROSCLEROSIS

Characteristics	Odds ratio	95 % CI	p value
HUA	2.513	1.379-2.513	0.003
Hypertension	2.118	1.211-3.707	0.009
DD (>72 mo)	2.599	1.468-4.601	0.001
Smoking	1.056	0.597-1.867	0.852
BMI (>25 kg/m^2)	1.204	0.691-2.097	0.512

With the rise of people's economic ability, the improvement of living standards and the change of lifestyle, more and more unreasonable diets high in calories, high in fat and low in fiber are being consumed, while physical work and exercise are increasingly lacking, and people's energy intake is far greater than their energy consumption, which promotes the occurrence of more and more metabolic diseases, and the incidence of diabetes is now in a state of continuous upward trend worldwide. The

incidence of diabetes is on the rise worldwide and China has become the world's largest country with diabetes.

T2DM is the main type of diabetes and diabetic macroangiopathy is a major complication of T2DM and an important cause of death and disability in patients with T2DM[8].

Atherosclerosis and atheromatous plaque formation are the main pathological basis of macrovascular disease and early detection and intervention of atherosclerosis is of great importance in the comprehensive management of T2DM[9]. Studies have confirmed that inflammatory factor-mediated inflammatory response, biochemical factors, blood pressure and age are all involved in the process of atherosclerosis from lipid pattern formation to final plaque rupture in T2DM patients, which is of great significance.

Inflammatory factors on atherosclerosis in T2DM patients are described here. Inflammatory cells migrate into the subendothelium and differentiate into macrophages, which then take up lipids and become foam cells, which are able to release various inflammatory mediators and promote the production and development of atheromatous plaques[10,11]. Inflammatory factors are mainly concentrated in CRP, TNF alpha (α), IL-1, IL-2, IL-6, IL-8, Plasminogen Activator Inhibitor-1 (PAI-1) and so on. Studies have shown that IL-6 can cause cell necrosis and promote endothelial damage, which further induces the expression of platelet-derived growth factor, TNF, macrophage colony-stimulating factor, etc., and promotes smooth muscle cell proliferation and insulin resistance and persistent hyperglycemia in T2DM patients are also involved in this inflammatory process. It has also been shown that IL-17 contributes to increased carotid atherosclerotic stenosis and leads to systemic vascular inflammation and increased plaque instability. Our study showed that, the inflammatory factors IL-1, IL-6 and TNF-α were significantly higher in the T2DM+atherosclerosis group compared to the control group, suggesting that IL-1, IL-6 and TNF-α are risk factors for carotid plaque in combination with T2DM, which is consistent with previous studies.

TG is an independent risk factor for the development of carotid atheroma in T2DM patients and the oxidative modification of LDL-C in the high glucose state directly damaged the endothelial cells; LDL-C also enhanced the adhesion of T lymphocytes and monocytes to endothelial cells[12]. LDL-C can also enhance the adhesion of T lymphocytes and monocytes to endothelial cells and induce the production of adhesion molecules; LDL-C is phagocytosed by macrophages and deposited on the arterial blood vessel wall, forming foam cells and further promoting the formation of atherosclerotic plaques. In contrast, HDL-C has a protective effect on atherosclerosis and the mechanism may be related to the intrinsic immune effect, with macrophages and endothelial cells being the main intrinsic immune effector cells. Our study showed that HDL-C was significantly reduced in the group with T2DM combined with carotid plaque and TG and LDL-C were significantly increased in the group with T2DM combined with carotid plaque, suggesting that HDL-C is a protective factor for T2DM combined with carotid plaque, while TG and LDL-C are risk factors for T2DM combined with carotid plaque.

The effect of SBP on atherosclerosis in patients with T2DM is as follows. Elevated blood pressure increases the shear force of blood flow on the endothelium and increases vasoactive substances such as endothelin and norepinephrine, resulting in damage to the endothelium and easy deposition of lipids in the damaged endothelium, which can lead to atherosclerosis and even plaque over time.

DKK4 acts not through activation of G protein-coupled signaling pathways, but through induction of beta (β)-inhibitory protein 2 aggregation, which in turn activates downstream signaling pathways or causes internalization of the receptor. DKK4 is an atypical chemokine receptor that increases cell growth and adhesion properties, not by activating G protein-coupled signaling pathways but by inducing β-inhibitory protein 2 aggregation, which in turn activates downstream signaling pathways or internalizes the receptor. The results showed significant macrophage accumulation and intimal hyperplasia in the damaged carotid arteries, while the experimental group was treated with C-X-C Chemokine Receptor Type 7 (CXCR7)-specific compound (CCX771), an artificial ligand for DKK4, and the hyperlipidemia-induced vascular injury and plaque formation were significantly improved. Our study reported that DKK4 expression was reduced in the serum of T2DM patients with normal carotid arteries and further reduced in the serum of patients with T2DM combined with carotid atheroma, suggesting that the decreased DKK4 level may

promote the early formation of carotid atheroma in type 2 diabetic patients and the development of atherosclerosis, and may be used as a predictor of carotid atherosclerosis in T2DM patients. We further investigated the relationship between DKK4 and the above-mentioned atherosclerosis. The results showed that DKK4 was positively correlated with HDL-C and negatively correlated with SBP, BMI, FBG, TG, IL-1, IL-6 and TNF-α. It is suggested that elevated SBP, increased blood glucose concentration, abnormal lipid metabolism and inflammatory status may be involved in influencing the alteration of serum DKK4 levels, thus contributing to the development of carotid atherosclerosis in T2DM patients.

The aim of this paper was to investigate the characteristics of serum DKK4 in T2DM patients and its relationship with factors affecting atherosclerotic plaque. The study suggested that decreased serum DKK4 levels may promote the formation of carotid atheroma in T2DM patients and monitoring its level may help in the early detection of macrovascular complications in diabetes. The results of this study also showed that DKK4 was positively correlated with HDL-C and negatively correlated with SBP, BMI, FBG, TG, IL-1, IL-6 and TNF-α. It suggested that elevated SBP, increased blood glucose concentration, abnormal lipid metabolism and inflammatory status may be involved in influencing the alteration of serum DKK4 levels, thus contributing to the development of carotid atherosclerosis in T2DM patients. Due to time and effort constraints, the present study did not further investigate the mechanisms associated with altered serum DKK4 expression levels in T2DM patients. Some studies have reported that DKK4 may improve the angiogenic function of Endothelial Progenitor Cells (EPCs) in diabetic limb ischemia through Protein kinase B (Akt)/Glycogen Synthase Kinase 3 Beta (GSK-3β)/proto-oncogene tyrosine-protein kinase (Fyn)-mediated activation of Nuclear factor erythroid 2-related factor 2 (Nrf2) and synergize with EPCs in the treatment of diabetic limb ischemia. We hope that future studies will provide better insight into the pathways and signaling pathways through which serum DKK4 affects the formation and development of atherosclerosis in T2DM patients, providing an idea for future elucidation of the etiology of T2DM, new clinical observables and therapeutic targets.

Conflict of interests:

The authors declared no conflict of interest.

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