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Clinical implication of alterations in serum Klotho levels in patients with type 2 diabetes mellitus and its associated complications☆

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ABSTRACT

Aim: To investigate the clinical significance of serum α-Klotho and β-Klotho levels in patients with type 2 diabetes mellitus (T2DM) and its associated complications.
Methods: Serum α-Klotho and β-Klotho levels were measured using an ELISA kit in 817 individuals, including 127 with T2DM, 106 with diabetic nephropathy, 99 with diabetic retinopathy, 108 with diabetic neuropathy, 102 with diabetic foot disease, 135 with T2DM and more than one complication and 140 healthy controls.
Results: Both α-Klotho and β-Klotho levels were significantly decreased in the T2DM group and the groups with associated complications compared with the levels in control group. The differences between the T2DM group and the T2DM with complications groups were not significant, except between the diabetic nephropathy group and the other diabetic complications groups. In addition, α-Klotho and β-Klotho levels were negatively correlated with serum fructosamine and HbA1c but were not associated with serum glucose in the model including all participants. Moreover, decreases in α-Klotho and β-Klotho levels in the high glucose-exposed cell culture model, which was dependent on glucose exposure time, were confirmed.
Conclusions: Levels of α-Klotho and β-Klotho were downregulated in patients in the T2DM and complications groups. Our findings indicate that serum Klotho levels were associated with the development of T2DM, and long-term control of blood glucose will be beneficial in ameliorating changes to α-Klotho and β-Klotho levels in patients with T2DM and complications.

1. Introduction

Diabetes is a group of diseases that are characterized by hyperglycemia and insulin defects and primarily includes type 1 and type 2 diabetes.[1] In addition, type 2 diabetes mellitus (T2DM), the most common type, accounts for >90% of clinical diabetes cases with a 2–4% prevalence worldwide, and usually occurs in people who are older than 40 years and obese.[1–3] Unfortunately, T2DM will often result in multiple complications, including manifestations in the kidneys, eyes, blood vessels, feet and nerves, which are the main causes of diabetes-related deaths.[4] Therefore, the need for a better understanding of the molecular mechanisms underlying the development of T2DM and its complications is imperative in order to develop more effective therapies.

Klotho is a type 1 trans-membrane protein that is particularly expressed in the kidney, where the ectodomain is shed and released into the systemic circulation as a soluble form.[5–7] In general, the Klotho family comprises three isoforms of the Klotho protein including α, β, and γ Klotho, which have pleiotropic functions in vivo.[8, 9] Studies suggest that α-Klotho may affect insulin and Wnt signaling, reduce oxidative stress, and regulate mineral homeostasis, while the major function of β-Klotho relates to metabolic regulation, glucose and fatty acid metabolism and bile acid synthesis.[10–13] However, the function of γ-Klotho remains largely elusive.[8]

More recent studies demonstrated that Klotho was involved in the development of T2DM, such as regulating glucose uptake, enhancing insulin sensitivity, attenuating oxidative stress and suppressing inflammation, which may affect the severity of T2DM.[14–16]In fact, evidence has shown that Klotho-induced inhibition of insulin signaling is associated with increased resistance to oxidative stress, which potentially contributes to the antiaging properties of Klotho.[11] Interestingly, T2DM is characterized by hyperglycemia, insulin resistance and high levels of oxidative stress.[1] A previous study showed that α-Klotho expression levels were decreased in an STZ-induced mouse model of diabetes and in patients with early diabetic nephropathy. Moreover another study demonstrated that Serum levels of α-Klotho and β-Klotho are downregulated in T2DM, diabetic nephropathy and diabetic coronary heart disease.[2, 17] Overall, the diagnostic role of the measurement of Klotho as a novel biomarker forT2DM may be relevant but warrants further research. However, few studies have focused on changes in the Klotho protein family in patients with T2DM who have serious complications.

☆ Conflicts of interest: None.

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Therefore, in this study, we systematically investigated the changes in serum α-Klotho and β-Klotho levels in patients with T2DM and associated complications and the correlation between α-Klotho and β-Klotho levels and disease severity in T2DM patients. In addition, we determined the association of changes in α-Klotho and β-Klotho levels with the laboratory indices reflecting glucose control, such as glucose, fructosamine, and HbA1c.

2. Materials and methods

2.1. Study population

This study was approved by the Ethics Committee of the Shantou University Medical College and was performed in accordance with the established national and institutional ethical guidelines regarding the involvement and collection of human samples for research. Written informed consent was obtained from all participants.

A total of 817 subjects of both sexes who were treated at the First Affiliated Hospital of Shantou Medical University from January 2016 to January 2018 were enrolled in our study. All subjects were diagnosed with T2DM with and without the corresponding complications by two chief endocrinologists according to the inclusion and exclusion criteria, which have been previously described,[18–20] and included the following categories: 127 individuals with T2DM, 106 with diabetic nephropathy, 99 with diabetic retinopathy, 108 with diabetic neuropathy, 102 with diabetic foot disease, and 135 with T2DM and more than one systemic complication. The healthy control group consisted of 140 individuals who visited in our hospital during the same period. We excluded patients who were <18 years old, were pregnant, had immunosuppressive disease or were receiving antihyperglycemic treatment, or had acute or chronic infection, cancer, other kidney diseases, other endocrine diseases, hepatic diseases, rheumatic diseases, retinopathy, or neuropathy. The NC group fulfilled the following criteria: (1) fasting serum glucose levels of 3.89–6.11 mmol/L, (2) HbA1c levels of 4–6%, (3) urinary albumin to creatinine ratio (UACR) of <30 mg/g, and (4) no history of diabetes, renal disease, or cardiovascular disease. The T2DM group diagnostic criteria include the following: (1) fasting serum glucose levels of ≥7.0 mmol/L, (2) HbA1c levels of ≥6.5%, (3) oral glucose tolerance test 2 h serum glucose levels of ≥11.1 mmol/L, (4) UACR of <30 mg/g, and (5) no history of renal disease, or cardiovascular disease. The criteria for the remaining groups with diabetic complication groups were based on those for T2DM, except UACR ≥ 30 mg/g in the diabetic nephropathy group, the retinal vascular lesions in the diabetic retinopathy group, spontaneous and evoked pain in the feet in the diabetic neuropathy group, and foot ulcers, deformity, infection, and gangrene in the diabetic foot group.

The general clinical and biochemical characteristics of the participants are presented in Table 1, including 127 individuals with T2DM (59 men, 68 women; median age: 52.5 years; range: 41 to 71 years), 106 with diabetic nephropathy (55 men, 51 women; median age: 54.6 years; range: 42 to 77 years), 99 with diabetic retinopathy (48 men, 51 women; median age: 52.6 years; range: 38 to 79 years), 108 with diabetic neuropathy (49 men, 59 women; median age: 53.2 years; range: 39 to 73 years), 102 with diabetic foot disease (58 men, 44 women; median age: 56.1 years; range: 42 to 79 years), and 135 with T2DM with more than one systemic complication (65 men, 70 women; median age: 54.4 years; range: 42 to 73 years). For comparison, a control group was established consisting of 140 healthy outpatients (77 men, 63 women; median age: 52.9 years; range: 39 to 71 years). TP, ALB, ALT, AST, TBIL, and DBIL were used to evaluate hepatic function; CREA and BUN, which are commonly used to assess renal function, were also used. The baseline characteristics of the controls and the other six groups showed no differences in the values for TP, ALB, ALT, AST, DBIL, TBIL, TG, TC, HDL, LDL, SBP, DBP and age ($P > 0.05$). However, significantly lower levels of HbA1c, fructosamine, glucose and BMI were found in the NC group than in the other groups. In addition, BUN quantities in the diabetic nephropathy group were significantly higher than those in the other groups ($P < 0.05$) with no significant differences found among the rest of the groups ($P > 0.05$). A similar trend was observed for serum CREA. Patients with a renal complication had significantly higher levels of CREA than those with no complication in the kidney ($P < 0.05$) with no significant differences found among the rest of the groups ($P > 0.05$).

Table 1
The study patients' clinical characteristics and laboratory investigations of the study patients.

	NC	T2D	Diabetic nephropathy	Diabetic retinopathy	Diabetic neuropathy	Diabetic foot	T2D with more than one complications	*P*
Sex (F/M)	63/77	68/59	51/55	51/48	59/49	44/58	70/65	NS
Age (years)	52.9 (39–71)	52.5 (41–71)	54.6 (42–77)	52.6 (38–79)	53.2 (39–73)	56.1 (42–79)	54.4 (42–73)	NS
SBP (mmHg)	112 ± 4.6	118 ± 4.6	118 ± 5.2	122 ± 5.2	124 ± 6.0	124 ± 6.2	129 ± 8.2	NS
DBP (mmHg)	71 ± 3.1	75 ± 3.5	72 ± 4.7	75 ± 4.4	72 ± 3.5	75 ± 4.1	74 ± 4.2	NS
BMI (kg/m^2)	22.1 ± 1.7	27.3 ± 3.6	27.5 ± 2.9	27.8 ± 3.0	27.9 ± 2.7	27.4 ± 2.1	28.1 ± 4.4	$^{*}P < 0.05$
TP (g/L)	77.2 ± 6.4	73.4 ± 6.0	75.1 ± 5.5	74.7 ± 6.0	73.9 ± 5.4	77.5 ± 5.1	74.5 ± 4.6	NS
ALB (g/L)	50.3 ± 2.6	49.9 ± 2.1	49.5 ± 2.2	49.7 ± 4.1	48.9 ± 3.1	50.0 ± 3.2	48.9 ± 2.9	NS
ALT (u/L)	27.9 ± 3.2	30.3 ± 2.3	31.8 ± 5.5	31.6 ± 3.1	30.4 ± 2.7	30.4 ± 2.0	30.1 ± 2.2	NS
AST (u/L)	24.4 ± 2.5	24.0 ± 2.1	25.1 ± 2.5	25.4 ± 2.1	24.2 ± 2.2	25.1 ± 2.4	26.1 ± 2.0	NS
TBIL (μmol/L)	10.4 ± 2.12	10.9 ± 2.42	10.1 ± 2.15	11.1 ± 2.05	11.3 ± 2.25	10.7 ± 2.83	11.7 ± 2.04	NS
DBIL (μmol/L)	3.8 ± 0.45	3.7 ± 0.33	3.5 ± 0.41	3.6 ± 0.31	3.5 ± 0.30	3.6 ± 0.28	3.4 ± 0.43	NS
TG (mmol/L)	0.94 ± 0.12	1.05 ± 0.24	1.17 ± 0.23	1.15 ± 0.17	0.99 ± 0.14	1.06 ± 0.14	1.16 ± 0.19	NS
TC (mmol/L)	4.0 ± 0.31	4.2 ± 0.33	4.1 ± 0.32	4.4 ± 0.31	4.2 ± 0.32	4.1 ± 0.30	4.2 ± 0.29	NS
HDL (mmol/L)	1.51 ± 0.24	1.54 ± 0.23	1.59 ± 0.22	1.57 ± 0.21	1.57 ± 0.30	1.48 ± 0.27	1.54 ± 0.23	NS
LDL (mmol/L)	3.1 ± 0.41	3.3 ± 0.42	3.0 ± 0.34	3.1 ± 0.29	3.2 ± 0.41	3.2 ± 0.30	3.0 ± 0.35	NS
BUN (mmol/L)	4.7 ± 0.44	5.2 ± 0.40	7.9 ± 0.89$^{\#}$	5.1 ± 0.37	5.2 ± 0.35	5.0 ± 0.37	5.1 ± 0.40	$^{\#}P < 0.05$
CREA (μmol/L)	89.7 ± 5.8	92.5 ± 6.0	169.8 ± 10.3$^{\#}$	92.0 ± 5.9	93.6 ± 4.7	93.1 ± 5.8	96.9 ± 7.9	$^{\#}P < 0.05$
Glucose (mmol/L)	5.07 ± 0.81	10.52 ± 5.05*	11.03 ± 6.89*	9.96 ± 6.07*	9.79 ± 5.96*	9.24 ± 6.49*	10.42 ± 5.67*	$^{*}P < 0.05$
fructosamine (mmol/L)	1.99 ± 0.14	2.43 ± 0.83*	2.47 ± 0.78*	2.57 ± 0.64*	2.66 ± 0.82*	2.65 ± 0.57*	2.49 ± 0.41*	$^{*}P < 0.05$
HbA1c (%)	5.55 ± 0.61	9.26 ± 1.66*	8.60 ± 2.02*	8.87 ± 1.39*	9.08 ± 2.12*	9.25 ± 2.10*	8.96 ± 2.04*	$^{*}P < 0.05$
α-Klotho (pg/ml)	715.90 ± 16.44	398.18 ± 36.67$^{*,\#}$	288.06 ± 43.28*	404.49 ± 35.17*	392.01 ± 52.15*	395.20 ± 53.17*	403.04 ± 31.87*	$^{\#,*}P < 0.001$
β-Klotho (pg/ml)	200.16 ± 10.73	82.62 ± 9.29$^{*,\#}$	57.17 ± 8.76*	80.36 ± 9.35*	81.54 ± 9.83*	80.70 ± 6.47*	77.47 ± 8.86*	$^{\#,*}P < 0.001$

Participant characteristics. Data are presented as the mean ± SD. $^{*}P < 0.05$ versus control, $^{\#}P < 0.05$ diabetic nephropathy versus T2DM, diabetic retinopathy, diabetic neuropathy, diabetic foot disease, T2DM with more than one systemic complication and nondiabetic healthy controls. *P*-values were calculated using Tukey's multiple comparisons test. One-way analysis of variance was used to compare the case groups and the control group. The same superscripted letters indicate statistical significance according to Tukey's multiple comparison test. Abbreviations: M: male; F: female; NC: nondiabetic healthy controls; T2DM: type 2 diabetes; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; AST: aspartate aminotransferase; ALT: alanine aminotransferase; TC: total cholesterol; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; TG: triglycerides; HbA1c: glycated hemoglobin; BUN: blood urea nitrogen; and CREA: serum creatinine.

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