Study of plasma klotho and plasma copeptin level in adolescents with type 1 diabetes mellitus and relation to microvascular complications

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Background
Type 1 diabetes, previously known as juvenile diabetes or insulin-dependent diabetes, is a form of diabetes characterized by the body's inability to produce insulin owing to the autoimmune destruction of the insulin-producing beta cells in the pancreas. Klotho (KL), known as an anti-aging protein, was discovered recently to have a multitude of biological effects. Major attention has been paid to the role of α-klotho (α-KL) in diabetes and its relation with diabetic nephropathy. The circulating form of α-KL, named as soluble KL, functions as an endocrine substance that exerts heterogeneous actions, including the modulation of renal function upon hyperglycemia, regulation of cell compensation, downregulation of inflammation and anti-oxidation effects.
Aim
The aim of this work is to study plasma KL and plasma copeptin in adolescents with type 1 diabetes and their relation to microvascular complications.
Patients and methods
This study was conducted in diabetes outpatient clinic in national institute of diabetes and endocrinology. The sample of 117 patients was divided into three groups: diabetes group, with 39 patients; diabetes with complication group, with 39 patients; and healthy controls, with 39 participants.
Results
Our study shows significant elevation of plasma copeptin level in type 1 diabetic adolescents, which was also significantly higher in the presence of microvascular complications particularly diabetic kidney disease (P=0.000). Although plasma KL is found to be significantly higher in type 1 diabetes without complications group, its level is dropped in the diabetes with complications group (P=0.005).
Conclusion
Plasma KL and plasma copeptin can be used as novel biomarkers for predicting early kidney injury in type 1 diabetic adolescents.

Keywords:
klotho, microvascular, plasma copeptin, type 1 diabetes mellitus

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Introduction

Type 1 diabetes is an immune-mediated depletion of β-cells, which leads to lifelong dependence on exogenous insulin. It can present with a variety of clinical symptoms, mostly catabolic symptoms, indicating insulin deficiency like polyuria, polydipsia, polyphagia, and persistent hyperglycemia. Children present with more acute symptoms like ketonemia and metabolic acidosis, and adults usually presents with a more gradual onset [1].

Klotho (KL), also known as an anti-aging protein, is expressed in multiple tissues with highest expression in the distal convoluted tubule of the kidney. It was proved that α-klotho (α-KL) has different functions. It is involved in regulation of parathyroid hormone release in the parathyroid gland, production of 1,25 (OH)2 vitamin D3, anti-oxidation, anti-apoptosis, promotion of angiogenesis, and vascularization and inhibition of fibrogenesis [2].

A lot of recent studies have focused on the role of α-KL in diabetes and its relation with diabetic kidney disease. Recently, major attention has been paid to the role of α-KL in diabetes and its relation with diabetic nephropathy. The circulating form of α-KL named as soluble KL acts as a hormone that is involved in modulation of renal function in response to

hyperglycemia, controlling cell compensation, reduction of inflammation, and anti-oxidation [2].

However, we still do not have enough information about the role of circulating α-KL levels in diabetes or diabetic nephropathy. There are two sides of the argument regarding KL role in diabetic kidney disease. Some research studies revealed that renal α-KL expression is markedly decreased in diabetic nephropathy in humans and rats [3,4]. On the contrary, some other studies claimed that there is no statistically significant difference in serum α-KL level between patients with diabetes without nephropathy and nondiabetic controls [5,6].

Arginine vasopressin (AVP), also known as antidiuretic hormone, is released in response to osmotic and nonosmotic stimuli and plays a key role in many physiologic and pathologic processes. The main function of AVP is the control of fluid homeostasis by inducing water conservation by the kidney, but it also stimulates arteriolar vasoconstriction and the release of adrenocorticotropic hormone. These actions are mediated by different AVP receptors located on various target cells. It is produced in the hypothalamus from a larger precursor (pre-pro-AVP). AVP is produced in equimolar amounts to copeptin, a glycopeptide with yet unknown biologic function. Copeptin remains stable in plasma, and its circulating concentrations correlate directly with those of AVP. Because AVP is unstable in isolated plasma or serum and its half-life is short, copeptin has become an easily measured surrogate marker reflecting vasopressin concentration [7]. The theory is that vasopressin might contribute to the progression of kidney damage and, thus, that plasma copeptin could be a good candidate for the identification of subjects at high risk for progression of nephropathy [8].

Aim of the work

The aim of this work is to study plasma KL and plasma copeptin in adolescents with type 1 diabetes and their relation to microvascular complications.

Patients and methods

Technical design

Study setting

This study was conducted in diabetes outpatient clinic in National Institute of Diabetes and Endocrinology.

Sample size

The sample was calculated to be 117 patients, who were divided into three groups:

(1) Diabetes group: 39 patients.
(2) Diabetes with complication group: 39 patients.
(3) Healthy controls: 39 patients.

Based on a previous study, the sample size determination was set before starting the study to a large number to obtain statistically significant results [9].

(1) Power of the study=80%.
(2) Confidence interval=95%.

Inclusion criteria

The following were the inclusion criteria:

(1) Adult patients of both sexes.
(2) Age 12–18 years old.
(3) Patients with type 1 diabetes (according to American Diabetes Association criteria).

Exclusion criteria

The following were the inclusion criteria:

(1) Known type 2 diabetic patients.
(2) Pregnant diabetic patients.
(3) Active inflammation.
(4) Active cancer.

Operational design

Type of study: this was a case–control study.

Tools: all participants were subjected to the following:

(1) Thorough history taking (including duration of diabetes, strategy of diabetes therapy, frequency of blood glucose monitoring, number of hypoglycemia episodes in the past 3 months, prevalence of retinopathy, and calculation of BMI).
(2) Systolic and diastolic blood pressure measurements.
(3) Routine laboratory investigation to fulfill inclusion and exclusion criteria:
    (a) Fasting blood sugar.
    (b) Glycated hemoglobin (HbA1c).
    (c) Urea and creatinine.
    (d) Albumin/creatinine ratio (A/C ratio).
    (e) Glomerular filtration rate (GFR) (will be calculated from serum creatinine).
    (f) Complete lipid profile.
(4) Blood samples were sent for testing of plasma copeptin and KL levels using ELISA.

Administrative design

A written informed consent was signed by each participant. The study has been approved from ethical committee of the hospital.

Results

There is no significant difference between the two diabetic groups regarding insulin regimen, short-acting insulin/day, long-acting insulin/day, and total insulin dosage/day.

There is also significant difference among the three groups regarding albuminuria and retinopathy. There is no significant correlation between plasma KL and plasma copeptin with other variables in the diabetes without complications group, except in the BMI.

There is no significant difference among the three groups regarding age, sex, weight, height, systolic/diastolic blood pressure, and number of hypoglycemia/week.

There is no significant difference among the three groups regarding blood urea nitrogen, total cholesterol, and low-density lipoprotein cholesterol. However, there is a significant difference regarding HbA1c, serum creatinine, A/C ratio, estimated glomerular filtration rate (eGFR), triglycerides, plasma copeptin, and plasma KL levels.

There is a significant difference among the three groups regarding plasma KL and plasma copeptin levels. Copeptin is higher in the diabetes without complications group compared with healthy controls, and it is significantly higher in the diabetes with complications group. Although KL is higher in the diabetes without complications group compared with healthy controls, its level starts to decrease in the diabetes with complications group (Table 1).

There was also no significant relation between plasma KL and plasma copeptin with age, height, weight, BMI, diabetes duration, insulin regimen, insulin dosage/day, HbA1c, systolic/diastolic blood pressure, numbers of hypoglycemia/week, blood urea nitrogen, total cholesterol, triglycerides, and A/C ratio. However, there is a significant relation between plasma KL/plasma copeptin and serum creatinine, eGFR, and low-density lipoprotein cholesterol. Moreover, there is significant correlation between plasma KL and plasma copeptin in diabetes with complications group (Table 2).

Our multivariate linear regression analysis showed that there is a significant positive relationship between plasma KL and plasma copeptin level with creatinine level and albumin/creatinine ratio and inverse correlation with GFR (Tables 3–5).

Table 1 Comparison among the three studied groups regarding sex, age, anthropometric measurements, blood pressure, frequency of hypoglycemia, and laboratory results

	Healthy controls	Diabetes without complications	Diabetes with complications	*P* value
Sex: male	15 (38.4)	12 (31.6)	26 (68.4)	0.454
Age	16±3.54	16.44±3.18	16.9±2.57	0.484
Weight (kg)	54.5 ±13.8	54.8±13.2	54.0±12.5	0.772
Height (m)	1.55 ±0.164	1.54±0.169	1.59±0.154	0.201
BMI	22.5 ±3.2	22.8±1.95	21.47±3.02	0.022
Systolic (mmHg)	110.3 ±12.4	111.28±11.4	110.0±12.87	0.644
Diastolic (mmHg)	75.6 ±10.1	72.44±8.5	71.54±10.1	0.672
Hypoglycemia (week)	None	1.15±1.33	1.49±1.54	0.309
HBA1c %	4.9±1.1	8.23±1.8	9.33±1.81	0.009*
BUN	15.51 ±2.98	15.58±2.77	15.75±3.07	0.805
Serum creatinine (mg/dl)	0.551 ±0.151	0.750±0.169	1.053±0.257	0.000*
A/C ratio	15±10.8	18.49±6.26	151.87 ±110.47	0.000*
eGFR	104 ±19.2	121.39±18.7	84.54±14.47	0.000*
Total cholesterol (mg/dl)	201 ±42.8	204.4±51.5	218.2±59.7	0.278
LDL cholesterol (mg/dl)	145.4 ±51.8	149.2±49.3	159.3±60.0	0.419
Triglycerides (mg/dl)	143 ±65.7	159.0±69.1	217.9±75.8	0.001*
Plasma copeptin	2.85 ±1.41	7.33±1.95	11.12±3.11	0.000*
Plasma klotho	0.895 ±0.49	2.81±1.95	2.41±0.788	0.005*

Data presents as mean±SD and *n* (%). A/C ratio; albumin/creatinine ratio; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein. *Means the result is statistically significant between different groups of the study.

Discussion

Our study shows significant elevation of plasma copeptin level in type 1 diabetic adolescents, which also become significantly higher in the presence of microvascular complications particularly diabetic kidney disease. In contrary to copeptin, plasma KL is found to be significantly higher in type 1 diabetes without complications group. KL level is decreased in the diabetes with complications group. So, copeptin and KL can be used as novel biomarkers for early diabetic kidney injury.

Table 2 Correlation between plasma copeptin and klotho and other variables in diabetes with complications group

	Plasma copeptin		Plasma klotho	
	Pearson correlation	Significance (2-tailed)	Pearson correlation	Significance (2-tailed)
Age	–0.373	0.020	0.150	0.363
Weight	–0.227	0.164	0.149	0.367
Height	–0.281	0.083	0.081	0.625
BMI	–0.017	0.920	0.096	0.563
Diabetes duration (years)	–0.111	0.503	0.007	0.967
Short-acting insulin (day)	–0.017	0.921	–0.281	0.083
Long-acting insulin (day)	–0.252	0.122	–0.209	0.201
Total insulin (day)	–0.199	0.224	–0.280	0.084
HBA1c %	0.024	0.887	0.140	0.394
Diastolic (mmHg)	–0.083	0.617	0.074	0.655
Systolic (mmHg)	0.158	0.335	0.215	0.189
Hypoglycemia (week)	–0.147	0.370	–0.149	0.364
BUN	0.037	0.823	–0.038	0.820
Serum creatinine	–0.950**	0.000	–0.284	0.080
A/C ratio	–0.154	0.350	0.264	0.105
eGFR	–0.965**	0.000	0.308	0.057
Total cholesterol	–0.206	0.209	0.265	0.104
LDL cholesterol	–0.212	0.194	0.350	0.029*
Triglycerides	–0.238	0.145	0.310	0.055
Plasma klotho	–0.336	0.036*	–	

A/C, albumin/creatinine ratio ; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; LDL, low-density lipoprotein. *Means the result is statistically significant between different groups of the study.

Table 3 Multivariate linear regression analysis of klotho and copeptin affection by creatinine increase

	Unstandardized coefficients			Standardized coefficients	*t*	Significance
	B	SE	95% confidence interval	Beta		
Constant	0.305	0.061	0.183–0.428	–	4.970	0.000
Copeptin	0.060	0.006	0.048–0.073	0.729	9.437	0.000
Klotho	0.019	0.007	0.024–0.043	0.107	3.386	0.017
			r^2=0.585			

Table 4 Multivariate linear regression analysis of klotho and copeptin affection by glomerular filtration rate decline

	Unstandardized coefficients			Standardized coefficients	*t*	Significance
	B	SE	95% confidence interval	Beta		
Constant	153.966	6.437	141.143–166.789		23.919	0.000
Copeptin	4.761	0.670	6.095–3.427	0.613	–7.109	0.000
Klotho	1.674	0.713	3.095–0.252	0.202	–2.346	0.022
			r^2=0.483			

Table 5 Multivariate linear regression analysis of klotho and copeptin influenced by albumin/creatinine ratio

	Unstandardized coefficients			Standardized coefficients	*t*	Significance
	B	SE	95% confidence interval	Beta		
Constant	105.906	15.606	136.994–74.818		6.786	0.000
Copeptin	8.082	1.624	4.848–11.317	0.252	4.978	0.000
Klotho	27.518	1.730	24.073–30.964	0.805	15.909	0.000
			r^2=0.822			

Regarding plasma copeptin level

Our study shows significant elevation of plasma copeptin level in type 1 diabetic adolescents, which also become significantly higher in the presence of microvascular complications particularly diabetic kidney disease.

Our study went with the results of Schiel and colleagues that there is an inverse association between plasma copeptin level and GFR, so copeptin can be used as a biomarker for renal function. There was also no significant difference in serum creatinine and eGFR between diabetics without complications group and healthy controls.

In contrary to our results, it was also found that there is no significant difference in copeptin concentration between healthy controls and diabetes without complications [10].

A similar study also found that plasma copeptin is significantly higher in type 1 diabetic patients with albuminuria compared with normoalbuminuric group, but it was limited only to men with type 1 diabetes [9].

The reason of vasopressin (and subsequently copeptin) elevation in diabetic individuals is not well understood. However, possible mechanisms include the following:

(1) Contraction of extracellular fluid volume induced by glycosuria.
(2) Augmented sensitivity of hypothalamic osmoreceptors in response to plasma osmolarity. Because osmoreceptors are stimulated by transcellular osmotic gradient, and glucose entrance to osmoreceptor cells is insulin dependent, hyperglycemia may be a powerful stimulus for thirst sensation and vasopressin secretion in type 1 diabetes [11].

Possible mechanism by which vasopressin causes kidney damage

The mechanism of kidney damage caused by elevated vasopressin (and copeptin) is still not well defined. Vasopressin-induced hyperfiltration eventually causes a vicious cycle that ultimately results in renal failure and end-stage renal disease. Vasopressin causes reduction of sodium concentration at macula densa because of vasopressin-dependent intrarenal handling of urea, which occurs by the following:

(1) Urea secretion in the pars recta.
(2) Reabsorption in the terminal collecting duct.
(3) Recycling in the medullary circulation.

All of these factors lead to elevated urea level at loop of Henle with consequent decrease of sodium level at macula densa, which ultimately leads to reduced tubulo-glomerular feedback stimulating the renin-angiotensin system and causing more increase in vasopressin secretion leading to a vicious circle [12].

Regarding plasma klotho level

In contrary to copeptin, plasma KL is found to be significantly higher in type 1 diabetes without complications group. KL level is dropped in the diabetes with complications group. So KL may be used as a novel biomarker for early diabetic kidney injury.

Our study went with the results of Lee *et al*. [13] that mentions that plasma KL is significantly elevated in diabetic patients with preserved renal functions, but it is inversely associated with albuminuria stages. Once diabetic patients develop albuminuria, KL level starts to drop.

A similar study suggested that soluble α-KL level is significantly reduced in young patient with chronic kidney disease, and with KL level decreasing, we could anticipate negative renal effect. Moreover, KL can be used as a marker for prediction of advanced kidney injury [4].

On the contrary, another study mentioned that there is no relation between soluble α-KL level and eGFR, and also arterial stiffness is raised in chronic kidney disease but was not related to KL level [14].Mechanism of renoprotective effect of KL is still not well understood. Its protective effect against soft tissue calcification might be a possible theory. Soft tissue calcification, particularly vascular calcification, is a common complication of chronic kidney disease, which occurs owing to hyperphosphatemia that can also fasten other chronic kidney disease complications such as hyperparathyroidism and renal osteodystrophy. KL has protective effect against soft tissue calcification by three different ways: phosphaturia, preservation of renal function, and direct effect on vascular smooth muscle cells by inhibiting phosphate uptake [15].

Conclusion

Plasma KL and plasma copeptin can be used as novel biomarkers for predicting early kidney injury in type 1 diabetic adolescents.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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