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Original Article

Biochemical changes correlated with blood thiamine and its phosphate esters levels in patients with diabetes type 1 (DMT1)

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Abstract: Thiamine (vitamin B1) is an essential enzyme cofactor in most organisms required at several stages of anabolic and catabolic intermediary metabolism. However, little is known on the positive effects of thiamine in diabetic type 1 (DMT1) patients. The objectives of this study were to evaluate the biochemical changes related to thiamine deficiency in patients with DMT1 outcomes among Saudi adults. We hypothesized that blood thiamine deficiency in patients with DMT1 manifestations might lead to an increase in metabolic syndrome. A total of 77 patients with DMT1 (age 35.8±5.5) and 81 controls (age 45.0±18.1) (total N = 158) were randomly selected from the Riyadh Cohort Study for inclusion. Saudi adults with diabetes type 1, a significant decrease in systolic ($P < 0.001$), and diastolic blood pressure ($P = 0.008$) and microalbuminuria ($P = 0.02$). Moreover, cholesterol, glucose and triglycerides were significantly increased (P 0.001, 0.001 and 0.008, respectively) in patients with diabetes type 1 compared to controls. On the other hand, HDL, TMP, TDP and thiamine, were significantly decreased in patients with diabetes type 1 (P 0.005, 0.002, 0.005, and 0.002), respectively. A strong association between blood thiamine level and diabetes type 1 was detected in our study population. The results confirmed the role of thiamine and thiamine phosphate esters, in preventing metabolic changes and complications of diabetes type 1. The levels of these thiamine and thiamine phosphate esters were correlated with diabetes related biomarkers including HDL, glucose, triglycerides and cholesterol, as well as microalbuminuria, LDL and urine thiamine. The results support a pivotal role of blood thiamine and its phosphate esters in preventing the biochemical changes and complications in patients with DMT1.

Keywords: Diabetes type 1 (DMT1), thiamine, HPLC, biochemical changes

Introduction

Diabetes mellitus (DM) is a significant public health burden due to its associated morbidity, economic costs and mortality. Glucose control as well as intensive management of blood pressure and cholesterol levels have been shown to improve health outcomes for patients with diabetes [1]. The prevalence and incidence of diabetes is increasing rapidly worldwide, including many Arab Gulf countries [2, 3]. According to a community-based national epidemiological health survey, the overall prevalence of diabetes mellitus in Saudi adults (age group of 30-70 years) is 23.7% [4]. A recent study by Al-Daghri et al. 2011 reported that the prevalence of diabetes mellitus in the kingdom of Saudi Arabia (KSA) was 31.6% [5].

Type I diabetes (formerly called insulin-dependent diabetes mellitus, or IDDM) is associated with an irreversible autoimmune destruction of the pancreatic islet beta-cells [6]. It is one of the most common metabolic disorders in children and adolescents, with increasing incidence and decreasing age of onset. It is characterized by insulin deficiency and hyperglycemia [6-10]. Therefore, patients depend entirely on daily insulin injection replacement therapy to regulate their blood glucose levels. The etiology

of type I diabetes is not completely understood, but it is recognized that genetic and environmental factors contribute to development and progression of the disease [11]. A worldwide increase in the incidence and variation (over 400-fold) of diabetes type 1 has been reported by the WHO Diabetes Mondiale (WHO DIAMOND) project group [12]. Moreover, Saudi Arabia is in the list of countries showing an increasing estimated prevalence of DMT1 from 1998-2010 [13]. A study performed by Al-Herbish et al. 2008 demonstrated that the prevalence DMT1 in Saudi Arabian children and adolescent was 109.5 per 100,000 [14].

Thiamine, a member of vitamin B family, cannot be synthesized by the human body and is required from exogenous sources to maintain a functional level. It is required for normal functions of the heart, muscles and nerves, and could be helpful to combat certain metabolic disorders [15]. The absorption of thiamine takes place via simple diffusion or trans-phosphorylation leading to thiamine monophosphate (TMP) and also through active transport, which accounts for the majority of thiamine absorption, leading to the phosphorylated product, thiamine diphosphate (TDP) [16], a necessary cofactor of enzymes related to carbohydrate metabolism. Mild thiamine deficiency or decreased plasma thiamine concentrations have been reported in diabetic patients [17-20]. Additionally, the risk of thiamine deficiency in type 2 diabetes was shown by an altered erythrocyte transketolase activity [21, 22]. However, there is disagreement in erythrocyte transketolase activity level while comparing diabetic and normal people [17, 21]. The studies also revealed mixed results with reduced [17, 18, 20], low [23] and normal [17] thiamine levels and its derivatives when measured in blood, serum and plasma. Our study demonstrates the relation between serum thiamine and its phosphate esters status in patients with DMT1.

Materials and methods

Study design and subjects

A number of seventy-seven Saudi patients with DMT1, aged 45±18 years old, and 81 healthy control subjects, aged 35±5.5 years old, have been selected randomly from subjects who participated in the on-going "Biomarker Screening" study of the Research Center for Biomarkers Research Laboratory in King Saud University, Riyadh, Saudi Arabia. Ethical approval was obtained from the College of Medicine and Research Center (CMRC) Ethics committee in King Khalid University Hospital and King Saud University Hospital, Riyadh, Kingdom of Saudi Arabia.

Anthropometrics

Anthropometry included height (rounded off to the nearest 0.5 cm) and weight (rounded off to the nearest 0.1 kg), which were measured using an appropriate international standard scale (Digital Person Scale; ADAM Equipment, Milford, CT, USA), as well as waist and hip circumference in centimeters, which were measured using a standard tape measure; body mass index (BMI) was calculated as kg/m^2. The Holtain Khan abdominal caliper by Holtain Ltd (Crymych, UK) was used to measure sagital abdominal diameter (SAD).

Biochemical parameters

Fasting blood was collected by an assigned physician at the primary healthcare centers. Blood was drawn, centrifuged and processed on the same day. Both whole blood and serum were placed in plain polystyrene tubes. Serum was delivered to Biomarker Research Program (BRP) for storage at -20°C. Fasting serum glucose levels, and complete lipid profile (triglycerides, total cholesterol, high density lipoprotein [HDL]-cholesterol) were determined using a biochemical analyzer (Konelab, Espoo, Finland). Low density lipoprotein (LDL)-cholesterol was calculated using the Friedman formula. This biochemical analyzer was calibrated routinely prior to the analysis of all serum samples using quality control samples provided by the manufacturer (Thermo-Fisher Scientific, Espoo, Finland). LINCOplex, human multiplex immunoassay kit based on Luminex 100 system platform (Luminex Corporation, Austin, TX, USA) was used for determination of three different panels by simultaneous detection of a great variety of cytokines, interleukins and immunoglobulins. The standard procedure protocol was followed. The concentrations of analyte in each sample were calculated with a five parameter model using Luminex IS software ver.2.3.

Blood thiamine analysis

A detailed methodology has been described previously. In brief, blood thiamine concentra-

Table 1. Clinical characteristics of patients with type 1 diabetes and healthy control subjects

	Control	Type 1 Diabetic	*P* value
N	81	77	
Age	35.8±5.5	45.0±18.1	< 0.001
BMI (kg/m^2)	25.6±5.0	26.4±4.8	0.28
Waist (cm)	83.4±15.0	91.8±19.5	0.005
Hips (cm)	97.1±18.1	99.9±19.6	0.34
Systolic BP (mmHg)	113.7±11.5	126.5±18.7	< 0.001
Diastolic BP (mmHg)	73.1±6.1	77.3±9.7	0.009
T.Cholesterol (mmol/l)	4.9±1.0	5.3±1.1	0.01
Glucose (mmol/l)	5.1±0.92	10.4±3.2	< 0.001
HDL (mmol/l)	0.84±0.33	0.66±0.41	0.005
Triglyceride (mmol/l)	1.4±0.81	1.9±0.91	0.008
LDL (mmol/l)	3.4±1.0	3.7±0.97	0.11
Thiamine (ng/ml)	4.1±2.1	3.6±1.5	0.25
TMP (ng/ml)	2.9±1.2	2.2±0.65	0.002
TDP (ng/ml)	35.9±14.7	27.7±15.1	0.005
T. Thiamine (ng/ml)	42.9±15.1	33.6±16.5	0.002
Thiamine_urine (µg/ml)	390.6 (227.6, 680.7)	446.7 (89.9, 1083.0)	0.79
Albumin_Serum (g/l)	48.7±4.7	46.1±6.2	0.04
Albumin_Urine (mg/l)	16.0 (6.0, 40.0)	28.6 (10.0, 527.0)	0.02
Serum Creatinine (µmol/L)	107.8±34.0	103.0±45.8	0.54
Creatinine_Urine (µmol/L)	6442.0 (3973.6, 14384.0)	9289.0 (73242.0, 10735.0)	0.03
Calcium (mmol/l)	2.4±0.33	2.6±0.41	0.01
Phosphorous (mmol/l)	1.3±0.48	1.4±0.29	0.21

Data represented by Mean ± standard deviation; Non Gaussian Variables represented by Median and interquartile.

tion, its phosphate esters and urine thiamine were quantified using HPLC-FLD [24]. Recovery studies were conducted after fortification to 0.002 and 0.01 mg L^{-1}, the last two values only for the pear samples. All spiked samples gave satisfactory recovery rates for the target analytes, the recovery values ranged from 91% to 112% for urine and from 95% to 115% for blood, respectively. All measurements were repeated after the 3rd and 6th month of thiamine supplementation.

Statistical analysis

All statistical analyses were carried out using SPSS version 16.0 (SPSS Inc, 1989-2007). Data was represented mean ± standard deviation. An independent sample t-test was used to compare the serum levels of various parameters between control and DMT1 subjects. Pearson correlations were used to establish relations between thiamine and its derivatives and anthropometric parameters and lipid profiles. Partial correlation was done adjusting for the potential confounders, such as age and BMI. *P* value less than 0.05 was considered statistically significant.

Results

Anthropometric and clinical parameters of Saudi adults with and without type 1 diabetes are summarized in **Table 1**. Serum concentrations of creatinine, cholesterol, triglycerides, and LDL cholesterol were within normal ranges in both the type 1 diabetes and control group. The systolic BP, diastolic BP, cholesterol, glucose, triglycerides, calcium and presence of microalbuminuria was significantly higher (P < 0.001, 0.009, 0.01, 0.001, 0.008, 0.01 and 0.02, respectively) as compared to controls. **Table 2** represents the overall comparison of thiamine and its derivatives (TMP, and TDP) with serum lipid profile and other biochemical parameters. Blood concentrations of thiamine and thiamine phosphate esters TMP and TDP were significantly lower in subjects with DMT1 (P 0.02). The urinary thiamine significantly was

Table 2. Correlation between thiamine and thiamine phosphate esters and measured biochemical parameters

	Thiamine		TMP		TDP		T. Thiamine	
	r	*P* value	r	*P* value	r	*P* value	r	*P* value
	158		158		158		158	
Waist (cm)	-0.04	0.64	0.01	0.97	-0.04	0.64	-0.05	0.62
Hips (cm)	-0.01	0.90	0.01	0.94	0.01	0.95	0.01	0.96
Systolic BP (mmHg)	0.06	0.53	-0.01	0.92	0.15	0.11	0.15	0.12
Diastolic BP (mmHg)	0.06	0.52	-0.01	0.93	0.14	0.16	0.14	0.16
T.Cholesterol (mmol/l)	-0.18	0.06	-0.07	0.50	-0.15	0.13	-0.16	0.09
Glucose (mmol/l)	0.01	0.93	-0.15	0.12	-0.28	0.004	-0.27	0.004
HDL (mmol/l)	-0.23	0.02	-0.03	0.75	-0.32	0.001	-0.34	0.001
Triglyceride (mmol/l)	0.06	0.53	0.18	0.06	0.03	0.73	0.05	0.58
LDL (mmol/l)	-0.11	0.27	-0.11	0.28	-0.15	0.13	-0.09	0.39
Thiamine_urine (µg/ml)	-0.12	0.38	-0.29	0.03	-0.55	< 0.001	-0.55	< 0.001
Albumin_Serum (g/l)	0.05	0.64	0.03	0.78	0.01	0.91	0.02	0.86
Albumin Urine (mg/l)	-0.16	0.22	-0.14	0.31	-0.21	0.11	-0.22	0.10
Serum Creatinine	-0.08	0.48	-0.08	0.44	-0.38	< 0.001	-0.37	0.001
Calcium (mmol/l)	-0.15	0.13	-0.12	0.22	-0.06	0.57	-0.08	0.42
Phosphorous (mmol/l)	-0.03	0.73	0.23	0.02	0.17	0.09	0.17	0.08

Partial Correlation done controlling for Age and BMI.

higher in subjects with DMT1 than healthy controls. A significant inverse association was found between thiamine, TDP and serum HDL level (TDP: r -0.41, P < 0.001; thiamine: r -0.42; P < 0.001). Additionally, a significant inverse association between TDP with glucose was observed (TDP: r -0.28, P 0.003; thiamine: r -0.25; P 0.008), urinary thiamine (TDP: r -0.58, P < 0.001; thiamine: r -0.51; P < 0.001), and serum creatinine (TDP: r -0.44, P < 0.001; Thiamine: r -0.47; P < 0.001). In case of TMP, no significant change was observed with any of the variables except phosphorous (r 0.22, P 0.02). In the DMT1 group, significant inverse relations of thiamine with serum HDL, urinary albumin, and serum creatinine are observed, while, TDP followed the same trend with HDL and urinary thiamine in addition to serum creatinine.

Discussion

We found that many biochemical markers related to diabetes type 1 showed highly significant differences between Saudi adults with and without diabetes type 1. Systolic Bp (P < 0.001), and diastolic Bp (P = 0.008) and urine microalbuminuria (P = 0.02) unformed in the diabetics. Cholesterol, serum glucose and triglyceride were significantly increased (P 0.001, 0.001 and 0.008), respectively in patients with diabetes type 1 compared to controls HDL, TMP, TDP and thiamine, on the other hand, were significantly decreased in patients with diabetes type 1 (P 0.005, 0.002, 0.005, and 0.002), respectively. A strong association between blood thiamine level and varies biochemical parameters were detected in diabetes type 1 in our population: these includes blood glucose, HDL and serum creatinine (P 0.008, 0.001 and 0.001), respectively. Data confirmed the role of thiamine and thiamine phosphate esters in preventing the metabolic changes and possibly the complications in subjects with diabetes type 1; levels of these thiamine and thiamine phosphate esters were correlated with diabetes, related biomarkers, including HDL, blood glucose, triglycerides and cholesterol as well as microalbuminuria, LDL and urinary thiamine.

Results of thiamine and its phosphate esters and urinary thiamine showed a negative correlation of blood thiamine and urinary thiamine excretion. Nutritional sufficiency of thiamine has also been defined on the basis of a threshold urinary excretion of thiamine greater than 0.20 mmol/24 h (> 60 mg/24 h) [25, 26]. On the basis of urinary excretion of thiamine, diabetic patients in two recent studies in the

United Kingdom and Pakistan had adequate nutritional intake of thiamine [17, 27]. To our knowledge, one study showed an inverse association of plasma thiamine concentration with renal clearance and fractional excretion of thiamine. Our results partially corroborate this finding supporting a negative correlation of blood TDP concentration with urinary thiamine level. Plasma thiamine concentration was lower in type 1 diabetes than controls as was urinary albumin excretion. Plasma thiamine concentration was decreased was 76% in type 1 and 75% in type 2 diabetic patients [17]. Decreased blood glucose with thiamine supplementation has been shown in DMT2 [28]. Our results showed a significant negative correlation between thiamine phosphate esters and blood glucose level in patients with diabetes type 1. The role of thiamine and its derivatives regarding the lipid profile is not clear and there have been contradictory observations reported. The concentration of plasma cholesterol and triglycerides were decreased by high dose of thiamine in diabetic rats without any effect on HDL level [29]. However, lower doses of thiamine are ineffective in combating these lipid profiles [30]. Serum concentration of cholesterol, glucose and triglyceride were significantly increased (P 0.001, 0.001 and 0.008), respectively in patients with diabetes type 1 compared to controls. HDL, TMP, TDP and thiamine, on the other hand, were significantly decreased in patients with diabetes type 1 (P 0.005, 0.002, 0.005, and 0.002), respectively. Negative correlation between blood thiamine, and its phosphate esters TMP and TDP with HDL levels were detected in diabetic group.

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Disclosure of conflict of interest

None.

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