Correlation of serum vitamin D level with ketoacidosis in Chinese children with type 1 diabetes mellitus

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Research Article

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Abstract

Objective: Diabetic ketoacidosis is a common complication in children with type 1 diabetes mellitus. The purposes of the present study were to explore clinical correlates of serum vitamin D level in Chinese children with type 1 diabetes.

Methods: A total of 143 inpatients (boys/girls = 60/83) were recruited from Tianjin Children's Hospital. Their demographic and clinical characteristics were collected. These patients were divided into the non-DKA group(n=43) and DKA group(n=100).

Results: The positive ZnT8-ab was significantly higher in DKA patients compared with non-DKA patients (p=0.038). There was a negative correlation between plasma glucose and the concentration of vitamin D(r =−0.188, p=0.024), although there was no significant difference in vitamin D between two groups of T1DM patients with or without DKA (p=0.317). The multiple logistic regression revealed that sex(male) and BMI were independent risk factors to predict the deficiency or insufficiency of Vitamin D in T1DM children. When BMI is lower than 16 kg/m^2 according to the cut-off value of the ROC curve, it provides some implications of Vitamin D deficiency or insufficiency in TIDM children (95%CI:0.534~0.721, P=0.014).

Conclusions: Our results suggested that positive ZnT8-ab was associated with a greater risk of DKA at T1DM onset. Additionally, neither vitamin D levels nor the proportion of patients with different levels of vitamin D differed between the two groups inT1DM children with or without DKA. Furthermore, Vitamin D level was negatively correlated with plasma glucose, lower BMI and male children with T1DM were prone to be deficient or insufficient of Vitamin D.

1 Background And Introduction

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease, especially in children with a higher occurrence rate. Diabetic ketoacidosis (DKA) is a potentially life-threatening complication of T1DM and is characterized by metabolic acidosis, hyperglycemia, and ketonaemia.[1]Insulin deficiency and increased levels of the counter-regulatory hormones (glucagon, catecholamines, cortisol, and growth hormone)can lead to DKA.[2]The clinical symptoms of DKA develop over several hours and include dehydration, nausea and/or vomiting, deep respiration, breath smells of acetone, blurry vision, drowsiness, eventually loss of consciousness (coma).[1] Evidence suggested that structured diabetes self-management education had led to DKA rates decrease.[3]However, recent data have shown that DKA is still a major cause of death in patients with T1DM.[4]

The development of T1DM involves many factors. The risk of DKA in established T1DM is 1–10% per patient per year. Newly diagnosed T1DM or patients with known diabetes have different risk factors for DKA.[2]A reviewed literature described potentially non-modifiable and modifiable risk factors for recurrent DKA, such as high HbA1c, low socioeconomic status, younger age.[1]All of these risk factors urgently

demand more efficient ways for early prediction, and eventually prevention or reversal of the disease as soon as possible. Biomarkers play an important role in clinical diagnosis, therapeutic effect, and prognosis, as indicators of normal and abnormal physiological or pathological processes.[5]

Now, autoantibodies (AAbs) may serve as relatively effective prognostic markers of the risk of diabetes progression.[6]However, the rate of development depends on many factors, including sex, age, genotype, type, and number of AAbs.[5] Hence, it is essential to find additional biomarkers that can predict the progression of T1DM and identify the best timing for DKA intervention and therapy. Autoimmune thyroid disease (AITD) and T1DM are two common autoimmune diseases and can occur concomitantly.[7]Many studies have demonstrated that changes in serum thyroid hormone parameters are associated with diabetes. A study concluded that thyroid hormone parameters seemed to reflect the effects of a prolonged period of catabolism, DKA, or both.[8] Besides, vitamin D is associated with both innate and adaptive immune systems. Deficiency or insufficiency of vitamin D seems to be a high-risk factor for autoimmune diseases such as T1DM and AITD.[7]

In this study, we aim to explore the relationship between vitamin D level and the risk of DKA and its components in patients with T1DM.

2 Materials And Methods

2.1 Subjects

This cross-sectional study was carried out in the Endocrine inpatient wards of Tianjin Children's Hospital, from June 1, 2017, to May 31, 2019. One hundred and forty-three children(boys/girls = 60/83) met the diagnosis of T1DM according to the WHO criteria, including fasting plasma glucose ≥ 7.0 mmol/l, 2-h postprandial plasma glucose ≥ 11.1 mmol/l, HbAlc ≥ 6.5%, as well as random plasma glucose concentration of ≥ 11.1 mmol/l. They were aged 10–180 months (mean age: 90.03 ± 43.07), with a mean age of onset of 74.52 ± 39.41 months (range 10–160 months). 143 patients were divided into two groups: T1DM without DKA group and T1DM with DKA group.

All the patients were obtaining medical treatment with insulin being subcutaneous injections such as Novolin and Novorapid. DKA was defined as blood glucose concentration > 13.9 mmol/l, Venous pH < 7.3 or serum bicarbonate (HCO_3) < 15 mmol/L, ketonemia or ketonuria, anion gap > 12. Major medical abnormalities, including central nervous system diseases, angiocardiopathy, or life-threatening medical illnesses (infections or cancer) were excluded. All subjects were Han Chinese.

After the study procedure was explained in detail to the parents of patients included in the study, they signed the informed consent document.

Before this study began, the research protocol was approved by the Institutional Review Board of Tianjin Children's Hospital.

2.2 Clinical and laboratory data

Bodyweight and height were measured in a standardized way and body mass index (BMI, weight in kg /square of height in meters) was calculated. Blood samples were collected before an initial insulin therapy. The plasma was separated, aliquoted, and stored at -70°C before use. Plasma glucose, HbA1c, C-peptide, triglyceride (TG), Total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), T3, T4, and TSH levels were measured at the diagnostic laboratory of Tianjin Children's hospital.

2.3 Vitamin D assays

The level of vitamin D was measured by equipment [API 3200MD™ LC/MS/MS System, AB Sciex]. It was defined as deficient, insufficient, and sufficient if vitamin D level was ≤ 15,≤20 > 15, and > 20 ng/ml, respectively.

2.4 Autoantibody assays

To confirm autoimmune diabetes origin, three kinds of typical autoantibodies were tested. The level of GAD-ab was determined by the GAD-Ab ELISA Kit, GAD-ab assay(positivity: >5u/ml). IA2-ab and ZnT8-ab were measured by the enzyme-linked immunosorbent assay([ElisaRSR™ IA-2 Ab Version 2, UK]/[ElisaRSR™ ZnT8 Ab™, UK]). Autoantibody for positivity were > 7.5u/ml and > 15u/ml for IA2-ab and ZnT8, respectively. The level of GAD-ab, IA2-ab, and ZnT8-ab were logarithmically transformed prior to analysis due to non-normal distributions.

2.5 Clinical measures

General information, sociodemographic characteristics, and family history of all subjects were collected by a researcher. Complete medical history, physical examination, and laboratory data were also obtained for each patient. Additional information was collected from available medical records and collateral resources.

2.6 Statistical analysis

Since all the demographic and clinical variables were normally distributed in subjects (Shapiro–Wilk one-sample test, all $p > 0.05$), comparisons of demographic and clinical data between two groups were analyzed by using independent samples t-tests for continuous variables and the chi-square test or Fisher's exact test for categorical variables. Analysis of covariance (ANCOVA) was further conducted to control for the effects of sex, age, and BMI on metabolic disturbances. Correlations between variables were assessed with Pearson's correlation coefficients. Bonferroni corrections were applied to each test to adjust for multiple testing. A binary logistic regression analysis was performed to predict risky variables. Finally, a multiple logistic regression analysis was used to identify significant predictive variables associated with Vitamin D. Statistical analyses were carried out using SPSS version 21, and MedCalc version 19.0.7 was used to draw ROC curves. $P < 0.05$ were considered indicative of statistical significance.

3 Results

3.1 Comparison of demographic and clinical variables between non-DKA and DKA patients

The average age of patients without DKA was 101.05 ± 41.92 months, and 85.16 ± 43.13 months in patients with DKA. The mean age of onset was younger in patients with DKA than in another group (70.09 ± 39.10 vs. 84.84 ± 38.61 months, p = 0.040). The mean BMI was 16.23 ± 2.15 and 15.52 ± 2.15 between non-DKA and DKA patients, respectively. Profiles of demographic and clinical variables of patients were summarized in Table 1.

Table 1
Demographic and clinical characteristics in patients with T1DM without or with DKA

Characteristics	T1DM without DKA (n = 43)	T1DM with DKA (n = 100)	t or X^2	P value
Age(months)	101.05 ± 41.92	85.16 ± 43.13	2.037	p = 0.044
Boys (%)	18/43(41.9)	42/100(42.0)		
Girls (%)	25/43(58.1)	58/100(58.9)	0.000	p = 0.988
BMI(kg/m2)	16.23 ± 2.15	15.52 ± 2.15	1.564	p = 0.121
Age of onset(months)	84.84 ± 38.61	70.09 ± 39.10	2.076	p = 0.040
Plasma glucose (mmol/l)	11.30 ± 6.15	23.54 ± 9.46	-9.187	p < 0.001
HbA1c (%)	9.40 ± 2.58	12.80 ± 2.27	-7.763	p < 0.001
C-peptide(nmol/l)	0.16 ± 0.19	0.13 ± 0.10	0.934	p = 0.355
25(OH)D(ng/ml)	21.41 ± 8.50	19.79 ± 8.97	1.005	p = 0.317
CRP(mg/L)	0.8	1.2	-1.139	p = 0.255
PCT(ng/ml)	0.05	0.08	-3.856	p < 0.001
K(mmol/l)	4.33 ± 0.49	4.50 ± 0.78	-1.317	p = 0.190
Ca(mmol/l)	2.44 ± 0.09	2.44 ± 0.15	0.267	p = 0.790
P(mmol/l)	1.47 ± 0.27	1.44 ± 0.37	0.570	p = 0.570
Mg(mmol/l)	0.82 ± 0.07	0.83 ± 0.10	-0.748	p = 0.456
Metabolites				
TG(mmol/L)	0.78 ± 0.39	4.03 ± 4.02	-8.003	p < 0.001

Abbreviations: T1DM,type 1 diabetes mellitus; DKA, Diabetic ketoacidosis; IL-6,interleukin-6;TG, triglyceride; TC, Total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol.

Characteristics	T1DM without DKA (n = 43)	T1DM with DKA (n = 100)	t or χ^2	P value
TC (mmol/L)	4.17 ± 0.89	5.24 ± 1.33	-5.510	p < 0.001
HDL-C(mmol/L)	1.88 ± 0.36	1.17 ± 0.41	9.134	p < 0.001
LDL-C(mmol/L)	2.29 ± 1.24	3.00 ± 1.84	-3.233	p = 0.002
Thyroid disease				
No (%)	28/43(65.1)	66/100(66.0)		
Yes (%)	15/43(34.9)	34/100(34.0)	0.010	p = 0.919
Family history of thyroid disease				
No (%)	40/43(93.0)	97/100(97.0)		
Yes (%)	3/43(7.0)	3/100(3.0)	0.401	p = 0.527
T3(nmol/L)	1.59 ± 0.39	0.99 ± 0.46	7.327	p < 0.001
T4(nmol/L)	106.63 ± 18.84	79.76 ± 36.76	5.661	p < 0.001
TSH(mIU/L)	2.15 ± 1.61	1.95 ± 1.89	0.595	p = 0.553
Family history of diabetes				
No (%)	30/43(69.8)	67/100(67.0)		
Yes (%)	13/43(30.2)	33/100(33.0)	0.106	p = 0.745
Peripheral neuropathy				
No (%)	33/43(76.7)	69/100(69.0)		
Yes (%)	10/43(23.3)	31/100(31.0)	0.882	p = 0.348
Autoantibodies				
GAD-ab (%)	65.0	75.0	0.752	p = 0.386

Abbreviations: T1DM,type 1 diabetes mellitus; DKA, Diabetic ketoacidosis; IL-6,interleukin-6;TG, triglyceride; TC, Total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol.

Characteristics	T1DM without DKA (n = 43)	T1DM with DKA (n = 100)	t or X^2	P value
lg(GAD-ab)(u/ml)	1.16 ± 1.10	1.44 ± 0.94	-1.116	p = 0.268
IA-2-ab (%)	45.0	60.0	1.371	p = 0.242
lg(IA-2-ab)(u/ml)	1.27 ± 0.90	1.63 ± 1.18	-1.451	p = 0.154
ZnT8-ab (%)	25.0	51.7	4.310	p = 0.038
lg(ZnT8-ab)(u/ml)	0.99 ± 0.90	1.27 ± 0.93	-1.646	p = 0.104
Abbreviations: T1DM,type 1 diabetes mellitus; DKA, Diabetic ketoacidosis; IL-6,interleukin-6;TG, triglyceride; TC, Total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol.				

Plasma glucose and HbA1c in patients with DKA were all higher than that of patients without DKA (all $p < 0.001$). There was no significant difference in C-peptide, CRP, K, Ca, P, Mg and Vitamin D level between the two groups (all $p > 0.05$). The DKA patients had significantly higher PCT, TG levels, TC levels, and LDL-C levels (all $p < 0.05$). In contrast, HDL-C level, T3 level, and T4 level were lower in patients with DKA (all $p < 0.001$). Analysis of covariance (ANCOVA) was further conducted to control for the effects of sex, age, and BMI on metabolic disturbances, significant differences still existed between the two groups. However, there is no significant difference in the prevalence of thyroid disease (34.9% vs. 34.0%), family history of thyroid disease (7.0% vs. 3.0%), family history of diabetes (30.2% vs. 33.0%), and peripheral neuropathy (23.3% vs. 31.0%) between non-DKA and DKA patients (all $p > 0.05$). There was no statistically significant difference in the concentrations of the three antibodies between the two groups. Whereas, the positive ZnT8-ab was significantly higher in DKA patients compared with non-DKA patients (25.0% vs. 51.7%, $p = 0.038$).

3.2 Comparison of the number of patients with the deficiency and insufficient of 25(OH)D between two groups

There was no significant difference in vitamin D level between the two groups of T1DM patients (21.41 ± 8.50 vs.19.79 ± 8.97, $p = 0.121$). We divided each group of patients into three subgroups according to the vitamin D level: deficient(≤ 15ng/ml), insufficient(≤ 20 > 15ng/ml), and sufficient(> 20 ng/ml). The proportion of non-DKA patients in each subgroup was 25.6% (11/43), 25.6% (11/43) and 48.8% (21/43),The proportion in each subgroup was 35.0% (35/100), 24.0% (24/100) and 41.0% (41/100) in DKA patients.The proportion of 25(OH) D deficiency in the DKA group was higher than that in the non-DKA group(35.0% vs.25.6%), although there was no significant difference($p = 0.526$). Comparison of serum 25(OH)D levels in two groups was shown in Table 2.

Table 2
Comparison of the number of patients with the deficiency and insufficient of 25(OH)D between two groups [n(%)]

	n	Deficiency of Vitamin D	Insufficiency of Vitamin D	Sufficient of Vitamin D	P value
T1DM without DKA	43	11(25.6%)	11(25.6%)	21(48.8%)	P = 0.526
T1DM with DKA	100	35(35.0%)	24(24.0%)	41(41.0%)	

3.3 Correlation analysis of serum 25(OH)D level and clinical measures

Pearson or Spearman correlation analysis showed that Vitamin D level were positive correlated with C-peptide ($r = 0.288$, $p = 0.001$), Ca ($r = 0.259$, $p = 0.002$),T3 ($r = 0.296$, $p < 0.001$) and T4 levels ($r = 0.294$, $p < 0.001$). Vitamin D level was inversely correlated with age ($r = -0.220$, $p = 0.008$), plasma glucose($r = -0.188$, $p = 0.024$) and PCT ($r = -0.235$, $p = 0.005$) in T1DM patients. However, no significant association was found between Vitamin D level and BMI, HbA1c, CRP, K, P, Mg, TG, TC, HDL-C, and TSH (all $p > 0.05$). Correlation analysis of serum 25(OH)D level and clinical measures were shown in Table 3.

Table 3
Correlation analysis of serum 25(OH)D level and clinical measures

	25(OH)D(ng/ml)	
Characteristics	r	P value
Age(months)	-0.220	P = 0.008
BMI(kg/m2)	0.136	P = 0.153
Age of onset(months)	-0.139	P = 0.099
Plasma glucose (mmol/l)	-0.188	p = 0.024
HbA1c (%)	-0.122	P = 0.146
C-peptide(nmol/l)	0.288	p = 0.001
CRP(mg/L)	-0.150	P = 0.073
PCT(ng/ml)	-0.235	P = 0.005
K(mmol/l)	-0.021	P = 0.803
Ca(mmol/l)	0.259	P = 0.002
P(mmol/l)	0.054	P = 0.524
Mg(mmol/l)	-0.142	P = 0.092
TG(mmol/L)	-0.142	P = 0.092
TC (mmol/L)	-0.156	P = 0.063
HDL-C(mmol/L)	0.044	P = 0.604
LDL-C(mmol/L)	-0.086	P = 0.312
T3(nmol/L)	0.296	p < 0.001
T4(nmol/L)	0.294	p < 0.001
TSH(mIU/L)	-0.081	P = 0.341

3.4 The clinical correlates of deficiency or insufficiency of serum 25(OH)D level in patients with T1DM

Using the scatter plot, we obtained a linear equation between blood glucose levels and vitamins D ($y=-0.22x + 24.3$, $R^2 = 0.035$).To explore the clinical correlates of deficiency or insufficiency of Vitamin D in patients with T1DM, we divided the patients into three subgroups. Statistically significant variables were shown in Table 4. Male patients with T1DM were prone to be deficient in Vitamin D (p = 0.006). A higher

level of plasma glucose and TG, and a lower level of C-peptide and T3 were more likely to lead to the deficiency or insufficiency of vitamin D (all $p < 0.05$).

Table 4
Demographic and clinical characteristics in patients with T1DM divided into 3 subgroups according to serum 25(OH)D level.

Variables	Deficiency of Vitamin D	Insufficiency of Vitamin D	Sufficient of Vitamin D	P value
Boys (%)	28/60(46.7)	13/60(21.7)	19/60(31.7)	
Girls (%)	18/83(21.7)	22/83(26.5)	43/83(51.8)	p = 0.006
BMI(kg/m2)	15.28 ± 1.94	15.24 ± 1.99	16.31 ± 2.31	p = 0.040
Plasma glucose(mmol/l)	22.87 ± 10.87	19.40 ± 11.03	17.88 ± 9.56	p = 0.041
C-peptide(nmol/l)	0.10 ± 0.95	0.13 ± 0.10	0.17 ± 0.16	p = 0.040
TG(mmol/L)	4.12 ± 4.67	2.90 ± 3.31	2.35 ± 2.82	p = 0.046
T3(nmol/L)	1.02 ± 0.49	1.09 ± 0.57	1.34 ± 0.57	p = 0.009

Binary logistic regression analyses found that males were 2.320 times more likely to be insufficient or deficient in Vitamin D compared with females((OR = 2.320, p = 0.017)). Moreover, the plasma glucose level in patients with insufficient or deficient Vitamin D was 1.035 times compared with those patients with sufficient Vitamin D(OR = 1.035, p = 0.046). Decreasing BMI (OR = 0.791, p = 0.014), C-peptide (OR = 0.043, p = 0.027),Ca(OR = 0.025, p = 0.010),T3(OR = 0.372, p = 0.004), and T4 (OR = 0.982, p = 0.001) levels were also important predictors for patients with the deficiency or insufficiency of Vitamin D.

3.5 Multivariate logistic regression to predict the deficiency or insufficiency of Vitamin D in T1DM patients

Finally, a multiple logistic regression was performed to predict the deficiency or insufficiency of Vitamin D in T1DM patients. Two variables statistically predicted the deficiency or insufficiency of Vitamin D in T1DM children, including sex and BMI. The coefficients of these variables were shown in Table 5.

Table 5
Predictors generated by Multivariate Logistic Regressionn with serum 25(OH)D level as dependent variables

	B	SE	OR	P value	95% CI for B
Sex	1.060	0.447	2.887	0.018	1.203 ~ 6.929
BMI	-0.269	0.108	0.765	0.013	0.619 ~ 0.945
Plasma glucose	0.022	0.025	1.022	0.386	0.973 ~ 1.074
C-peptide	-3.224	1.761	0.040	0.067	0.001 ~ 1.255
Ca	-1.175	1.763	0.309	0.505	0.010 ~ 9.770
T_3	-0.358	0.486	0.699	0.461	0.270~-1.811
(constant)	7.370	4.633	1587.347	0.112	-

3.6 ROC curve of BMI predicting the deficiency or insufficiency of Vitamin D in T1DM patients

ROC curve of BMI predicting the deficiency or insufficiency of Vitamin D in T1DM children was shown in Fig. 1. ROC curve showed that BMI presented 52.1% specificity and 71.4% sensitivity and the area under the curve (AUC) was 0.631. When the level of BMI is lower than 16 kg/m2 according to the cut-off value, it provides some implications and warnings to the risk of Vitamin D deficiency or insufficiency in TIDM children(95%CI:0.534 ~ 0.721,P = 0.014).

4 Discussion

Our study confirmed that ZnT8-ab positivity was associated with a greater risk of DKA in T1DM children. Moreover, neither vitamin D levels nor the proportion of patients with different levels of vitamin D differed between the two groups of children with T1DM. Besides, Vitamin D level was negatively correlated with plasma glucose, lower BMI and male children with T1DM were prone to be deficient or insufficient of Vitamin D.

We found that patients with DKA were younger than those without DKA, not only age of onset but also current age, which can be defined by a consensus statement from ISPAD.[2]A review considered that AAbs were regarded as the gold standard for the prediction of T1DM progression currently.[5]In our study, we observed the prevalence of positive ZnT8-ab was at greater risk of DKA, which was in concordance with that found in the study performed by Niechcial et al. Interestingly, they found that the titers of GAD-ab and

IA2-ab were lower in DKA patients but titer of ZnT8-ab was higher in DKA patients.[6]However, based on our results, the titers of the three AAbs (GAD-ab, IA2-ab, and ZnT8-ab) weren't a significant difference between two groups. The research demonstrated that ZnT8-ab positive patients had a higher incidence of multiple diabetes-related AAbs, which means more severe β-cell dysfunction and higher prevalence of DKA.[9]ZnT8 is a 369-amino acid pancreas-specific zinc transporter, which was encoded by the SLC30A8 gene at the chromosome 8q14.11, and SLC30A8 is a major target of humoral autoimmunity in T1DM.[10]ZnT8 plays a key role in glucose homeostasis. A study suggested that ZnT8 was a crucial protein for both zinc accumulation and regulation of insulin secretion in pancreatic β cells.[11]Compared with other autoimmune markers, ZnT8-ab is highly βcell-specific and ZnT8-ab expression may not occur until there is enough β-cell damage.[9] In hence, positive ZnT8-ab can be used as an indicator to predict the progression of T1DM.

In our study, we found a lower level of T3 in DKA patients. As we all know that patients with diabetes have a high risk of AITD.[12] 49 of 143 T1DM patients in our study suffered from thyroid disease, such as multiple cystic nodules of the thyroid, hypothyroidism, Autoimmune Polyendocrine Syndrome. The pathogenesis of AITD involves cellular and humoral autoimmune mechanisms against the thyroid gland. T lymphocytes infiltrate the glands, then subsequent development of various degrees of thyroid dysfunction.[7] Thyroid hormones T4 and T3 were produced by a glycosylated transmembrane protein named thyroid peroxidase, which is distributed in the apical part of follicular thyroid cells.[13]Metabolomics techniques have shown that people who develop diabetes have different levels of certain lipids when compared with people who remain non-diabetic.[14]In addition, thyroid dysfunction can lead to metabolic disorders. This in turn results in stimulation of glycogenolysis and gluconeogenesis, increased glucose absorption, and lipolysis, causing deterioration of metabolic control.[15]Anyway, recognition of these metabolic alterations may aid in studies of disease progression and may open a time window for DKA prevention strategies.

Currently, many studies are exploring the relationship between vitamin D and immune-related diseases, including T1DM.[16] Because of the discovery of specific vitamin D receptors (VDR) on pancreatic β-cell, the role of active vitamin D on β-cell' function was confirmed.[17]There was evidence suggested that calcitriol maintained β-cell mass and improves islets function via several pathways. The insulin secretion process was a calcium-dependent mechanism.[18]A review concluded that vitamin D improves glucose homeostasis, and the molecular mechanism is that promoting insulin sensitivity via at least two different pathways: promoting β-cell function by ameliorating deleterious molecular mechanisms and increasing peripheral insulin sensitivity.[19]VDR expression and activity were important for all stages of T cells, ranging from development to differentiation and elicitation. Administration of $1,25(OH)_2D_3$ can enhance Treg and inhibit autoreactive T_H1, leading to a reduction in the incidence of T1DM in the diabetic mouse model.[17]However, we didn't find a significant difference in vitamin D level between the two groups in T1DM patients. And vitamin D may not forecast the incidence of DKA in T1DM patients. Similarly, recent literature had questioned the protective effect of vitamin D.[20]Multiple trials have failed to demonstrate the

obvious benefits of vitamin D supplementation for diabetes people.[21] A RCT trial concluded that vitamin D3 supplementation at a dose of 4000 IU/d didn't result in a significantly lower risk of diabetes than placebo.[22]In despite this, we found that vitamin D levels were negatively correlated with plasma glucose levels in patients with T1DM. Diabetes can contribute to the deficiency of insufficiency of vitamin D, which is consistent with a review.[23]Various factors, including sex, age, nutrition status, location, and physical fitness, affected vitamin D status.[23]In our study, male patients with T1DM were prone to be deficient or insufficient of Vitamin D. A recent study concluded that Vitamin D insufficiency was highly prevalent among T1DM children of Central Florida and statistically significant correlation was found between vitamin D status and BMI. There was a significant association between vitamin D deficiency and BMI ($p = 0.035$), which was similar to our conclusion.[24]

Several limitations of our study should be noted.

Firstly, this study was a cross-sectional design and all subjects were recruited from a local hospital. Hence, observations and conclusions should be treated with caution. Secondly, the concentration of Vitamin D was measured at disease onset. There is no uniform time point of Vitamin D measurements, in winter or summer time. Therefore, further accurate studies need to be conducted to validate our findings. Thirdly, we had a comparatively small sample size of patients, which had become smaller when dividing into subgroups. Therefore, the results and conclusions in our study should be regarded as preliminary and should be confirmed after further prospective, multicenter, and large-scale trials.

5 Conclusion

In summary, the current study has shown that ZnT8-ab can emerge as a reliable marker for DKA in T1DM patients. Additionally, neither vitamin D levels nor the proportion of patients with different levels of vitamin D differed between the two groups of children with T1DM. Furthermore, Vitamin D level was negative correlated with plasma glucose. Lower BMI, and male children with T1DM were prone to be deficient or insufficient of Vitamin D. Further prospective and multicenter studies including large population size, more parameters, and more ethnic groups are needed to demonstrate these findings.

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Tables

Table 1 Demographic and clinical characteristics in patients with T1DM without or with DKA

Characteristics	T1DM without DKA (n=43)	T1DM with DKA (n=100)	t or χ^2	P value
Age(months)	101.05±41.92	85.16±43.13	2.037	p=0.044
Boys (%)	18/43(41.9)	42/100(42.0)		
Girls (%)	25/43(58.1)	58/100(58.9)	0.000	p=0.988
BMI(kg/m2)	16.23±2.15	15.52±2.15	1.564	p=0.121
Age of onset(months)	84.84±38.61	70.09±39.10	2.076	p=0.040
Plasma glucose (mmol/l)	11.30±6.15	23.54±9.46	-9.187	p<0.001
HbA1c (%)	9.40±2.58	12.80±2.27	-7.763	p<0.001
C-peptide(nmol/l)	0.16±0.19	0.13±0.10	0.934	p=0.355
25(OH)D(ng/ml)	21.41±8.50	19.79±8.97	1.005	p=0.317
CRP(mg/L)	0.8	1.2	-1.139	p=0.255
PCT(ng/ml)	0.05	0.08	-3.856	p<0.001
K(mmol/l)	4.33±0.49	4.50±0.78	-1.317	p=0.190
Ca(mmol/l)	2.44±0.09	2.44±0.15	0.267	p=0.790
P(mmol/l)	1.47±0.27	1.44±0.37	0.570	p=0.570
Mg(mmol/l)	0.82±0.07	0.83±0.10	-0.748	p=0.456
Metabolites				
TG□mmol/L)	0.78±0.39	4.03±4.02	-8.003	p<0.001
TC (mmol/L)	4.17±0.89	5.24±1.33	-5.510	p<0.001
HDL-C(mmol/L)	1.88±0.36	1.17±0.41	9.134	p<0.001
LDL-C(mmol/L)	2.29±1.24	3.00±1.84	-3.233	p=0.002
Thyroid disease				
No (%)	28/43(65.1)	66/100(66.0)		
Yes (%)	15/43(34.9)	34/100(34.0)	0.010	p=0.919
Family history of thyroid disease				
No (%)	40/43(93.0)	97/100(97.0)		
Yes (%)	3/43(7.0)	3/100(3.0)	0.401	p=0.527
T3(nmol/L)	1.59±0.39	0.99±0.46	7.327	p<0.001
T4(nmol/L)	106.63±18.84	79.76±36.76	5.661	p<0.001
TSH(mIU/L)	2.15±1.61	1.95±1.89	0.595	p=0.553
Family history of diabetes				
No (%)	30/43(69.8)	67/100(67.0)		
Yes (%)	13/43(30.2)	33/100(33.0)	0.106	p=0.745
Peripheral neuropathy				
No (%)	33/43(76.7)	69/100(69.0)		
Yes (%)	10/43(23.3)	31/100(31.0)	0.882	p=0.348

Autoantibodies				
GAD-ab (%)	65.0	75.0	0.752	p=0.386
Ig(GAD-ab)(u/ml)	1.16±1.10	1.44±0.94	-1.116	p=0.268
IA-2-ab (%)	45.0	60.0	1.371	p=0.242
Ig(IA-2-ab)(u/ml)	1.27±0.90	1.63±1.18	-1.451	p=0.154
ZnT8-ab (%)	25.0	51.7	4.310	p=0.038
Ig(ZnT8-ab)(u/ml)	0.99±0.90	1.27±0.93	-1.646	p=0.104

Abbreviations: T1DM,type 1 diabetes mellitus; DKA, Diabetic ketoacidosis; IL-6,interleukin-6;TG, triglyceride; TC, Total cholesterol; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol.

Table 2 Comparison of the number of patients with the deficiency and insufficient of 25(OH)D between two groups [n(%)]

	n	Deficiency of Vitamin D	Insufficiency of Vitamin D	Sufficient of Vitamin D	P value
T1DM without DKA	43	11(25.6%)	11(25.6%)	21(48.8%)	P=0.526
T1DM with DKA	100	35(35.0%)	24(24.0%)	41(41.0%)	

Table 3 Correlation analysis of serum 25(OH)D level and clinical measures

Characteristics	25(OH)D(ng/ml)	
	r	P value
Age(months)	-0.220	P=0.008
BMI(kg/m2)	0.136	P=0.153
Age of onset(months)	-0.139	P=0.099
Plasma glucose (mmol/l)	-0.188	p=0.024
HbA1c (%)	-0.122	P=0.146
C-peptide(nmol/l)	0.288	p=0.001
CRP(mg/L)	-0.150	P=0.073
PCT(ng/ml)	-0.235	P=0.005
K(mmol/l)	-0.021	P=0.803
Ca(mmol/l)	0.259	P=0.002
P(mmol/l)	0.054	P=0.524
Mg(mmol/l)	-0.142	P=0.092
TG(mmol/L)	-0.142	P=0.092
TC (mmol/L)	-0.156	P=0.063
HDL-C(mmol/L)	0.044	P=0.604
LDL-C(mmol/L)	-0.086	P=0.312
T3(nmol/L)	0.296	p<0.001
T4(nmol/L)	0.294	p<0.001
TSH(mIU/L)	-0.081	P=0.341

Table 4 Demographic and clinical characteristics in patients with T1DM divided into 3 subgroups according to serum 25(OH)D level.

Variables	Deficiency of Vitamin D	Insufficiency of Vitamin D	Sufficient of Vitamin D	P value
Boys (%)	28/60(46.7)	13/60(21.7)	19/60(31.7)	
Girls (%)	18/83(21.7)	22/83(26.5)	43/83(51.8)	p=0.006
BMI(kg/m2)	15.28±1.94	15.24±1.99	16.31±2.31	p=0.040
Plasma glucose(mmol/l)	22.87±10.87	19.40±11.03	17.88±9.56	p=0.041
C-peptide(nmol/l)	0.10±0.95	0.13±0.10	0.17±0.16	p=0.040
TG(mmol/L)	4.12±4.67	2.90±3.31	2.35±2.82	p=0.046
T3(nmol/L)	1.02±0.49	1.09±0.57	1.34±0.57	p=0.009

Table 5 Predictors generated by Multivariate Logistic Regressionn with serum 25(OH)D level as dependent variables

	B	SE	OR	P value	95% CI for B
Sex	1.060	0.447	2.887	0.018	1.203~6.929
BMI	-0.269	0.108	0.765	0.013	0.619~0.945
Plasma glucose	0.022	0.025	1.022	0.386	0.973~1.074
C-peptide	-3.224	1.761	0.040	0.067	0.001~1.255
Ca	-1.175	1.763	0.309	0.505	0.010~9.770
T_3	-0.358	0.486	0.699	0.461	0.270~-1.811
(constant)	7.370	4.633	1587.347	0.112	-

Figures

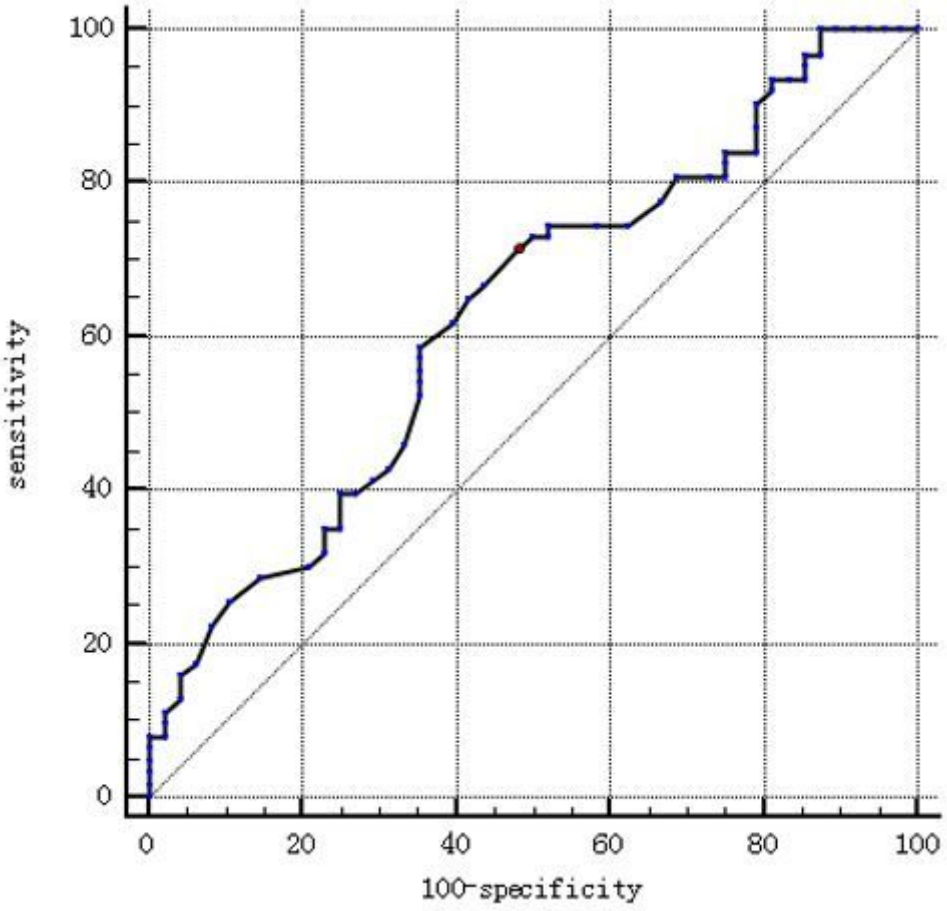

Figure 1

ROC curves of BMI predicting the deficiency or insufficiency of Vitamin D in T1DM patients

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