Vitamin D levels are associated with low-density lipoprotein cholesterol in Chinese children with type 1 diabetes

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Abstract

Objective There is an increased level of low-density lipoprotein cholesterol (LDL-C) in children with type 1 diabetes mellitus (T1DM). In addition, the Vitamin D level in T1DM patients is usually below the normal reference range. The aim of this study was to explore the relationship between Vitamin D levels and LDL-C in Chinese children with T1DM.

Methods A retrospective cross-sectional study was conducted in the Endocrine inpatient wards of Tianjin Children's Hospital, 143 children with T1DM were included. The related clinical and laboratory examinations, including anthropometric parameters, lipid profiles, and Vitamin D levels, were collected in all subjects.

Results The univariate analysis results did not show a significant correlation between Vitamin D levels and LDL-C (P=0.634). Furthermore, a nonlinear relationship was observed between Vitamin D levels and LDL-C by smooth curve fitting after adjusting for potential confounders. A multivariate piecewise linear regression model revealed a significant negative association between LDL-C and Vitamin D levels when LDL-C was greater than 3.1 mmol/L (β = -2.9, 95% CI -5.4, -0.5; P=0.022). However, we did not observe a significant relationship between LDL-C and Vitamin D levels when LDL-C was lower than 3.1 mmol/L (β = 2.4, 95% CI -0.2, 5.1; P=0.076).

Conclusions This study identified a nonlinear relationship between Vitamin D levels and LDL-C independent of other potential confounding factors, suggesting that the deficiency or insufficiency of Vitamin D in T1DM children with high LDL-C levels should be considered, especially LDL-C is higher than 3.1 mmol/L, which provides evidence of the timing about Vitamin D supplementation in T1DM children.

1. Introduction

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease characterized by insulin deficiency, especially in children with a higher occurrence rate[1]. In recent decades, the mortality from diabetes-related cardiovascular disease has increased significantly. Some studies have provided evidence that diabetes is a risk factor associated with cardiovascular disease[2, 3]. As a major risk factor for the progression of atherosclerosis, dyslipidemia may contribute to the excess risk of cardiovascular events in patients with diabetes[4]. Lipidomics studies of patients that progressed to clinical disease revealed that some classes of lipids show dysregulation in the blood. Based on the lipid management recommendations, low-density lipoprotein cholesterol (LDL-C) is the main target in the treatment of diabetic dyslipidemia[5].

The accumulation of LDL-C into fatty streaks in the intima of blood vessels, which is the earliest morphological change that occurs during the development of atherosclerosis[6]. While LDL-C reduces the release of NO, HDL-C stimulates the production of NO and inhibits the adhesion of monocytes to endothelium, which is related to the protective effect[7]. Previous prospective cohort studies have
suggested that compared to other lipid profiles, LDL-C increased in childhood is the strongest predictor for the risk of dyslipidemia and cardiovascular disease in adulthood[8, 9]. The deficiency or insufficiency of Vitamin D levels in T1DM children is highly prevalent[10]. Immunologic effects of Vitamin D on human health and disease have been demonstrated[11]. But the link between Vitamin D levels and LDL-C has not received much attention. Considering the high prevalence of vitamin D deficiency among T1DM children, its potential health implications, and its convenience of treatment, it is necessary to consider regularly screening T1DM children for vitamin D insufficiency or deficiency[10].

Up to date, the relationship between Vitamin D levels and LDL-C was studied rarely in children with T1DM. Hence, we gathered the lipid profiles of children with T1DM at a single center in China and explored the relationship between LDL-C and Vitamin D levels in this population.

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, HbA1c ≥ 6.5%, as well as random plasma glucose concentration of ≥ 11.1 mmol/L. Major medical abnormalities, including central nervous system diseases, angiocardiopathy, or life-threatening medical illnesses (infections or cancer) were excluded. All subjects were Han Chinese. They were aged 10 months –15 years (mean age: 7.50 ± 3.60 years), with a mean age of onset ranged 10 months–14 years. 143 patients were divided into three groups according to the Vitamin D levels: deficient(≤ 15ng/ml), insufficient(≤ 20 >15ng/ml), and sufficient(>20 ng/ml).

All the patients were obtaining medical treatment with insulin being subcutaneous injections such as Novolin and Novorapid. We collected general information and sociodemographic characteristics of all subjects from available medical records and collateral resources.

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 biochemical measurements

Bodyweight and body height were recorded to the nearest 0.1 kg and 0.1 cm, respectively, while subjects were wearing light indoor clothing without shoes. Body mass index (BMI) was calculated as the weight in kilograms divided by the height in meters squared (kg/m2). BMI SDS values were calculated according to development figures for Chinese children and teenagers published in 2009[12].

Venous blood samples, before an initial insulin therapy, were collected for laboratory tests after overnight fasting of at least 10 h. The plasma was separated, aliquoted, and stored at -70 °C before use.
Concentrations of fasting plasma glucose (FPG) were measured by the enzymatic hexokinase method. Hemoglobin A1c (HbA1c) was assessed by a Special protein gold standard detector [AS100, Axis-shields, Norway]. C-peptide levels were measured using an automatic biochemistry analyzer [(Cobas 8000, e602, Roche, China)]. Triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein A (Apo A), and apolipoprotein B (Apo B) were measured using an automatic biochemistry analyzer [(Cobas 8000, c701, Roche, China)] at the testing laboratory of Tianjin Children’s hospital. Non-HDL-C levels were calculated from the difference between serum TC and HDL-C. LDL-C dyslipidemia was defined according to the expert consensus of Chinese children and adolescent dyslipidemia.[13]

2.3. Vitamin D assays

The level of Vitamin D was measured by equipment [API 3200MD™ LC/MS/MS System, AB Sciex] and 25(OH)D was determined by high-performance liquid chromatography-UV detection. It was defined as deficient, insufficient, and sufficient if Vitamin D levels were ≤15, ≤20 >15, and >20 ng/ml, respectively.

2.4. Statistical analysis

The statistical analyses were performed using SPSS version 21, EmpowerStats Software (http://www.empowerstats.com, X&Y Solutions, Inc., Boston, MA) and R (http://www.R-project.org). Continuous variables are presented as the means ± SD except for skewed variables, which were presented as medians (interquartile ranges). Categorical variables are expressed as proportions. Comparisons of demographic and clinical variables between three groups were performed with ANOVA analysis or the chi-square test. Bonferroni corrections were applied to each test. A univariate analysis was used to determine the association between Vitamin D levels and LDL-C as well as the other independent variables. We then investigated the relationship between Vitamin D levels and LDL-C using smooth curve fitting after adjusting for potential confounders. Finally, we further applied a multivariate piecewise linear regression model to assess the threshold association between Vitamin D levels and LDL-C. All statistical tests were two-sided, and P < 0.05 were considered statistically significant.

3. Results

3.1. Demographic and clinical characteristics in patients with T1DM divided into 3 groups according to serum Vitamin D levels

A total of 143 inpatients with T1DM (boys/girls = 60/83) were recruited from the Endocrine inpatient wards of Tianjin Children’s Hospital. In general, the average age of the 143 selected subjects was 7.5±3.6 years old. No statistically significant differences were detected in age, age of onset, HbA1c, CRP, PCT, TC, HDL-C, LDL-C, Non-HDL-C, ApoA, ApoB, Lipoprotein, and Free fatty acid among the different groups (all p>0.05). There were significant differences in sex, BMI, BMI SDS, FPG, C-peptide, and TG among the different groups (all p<0.05). Demographic and clinical characteristics of T1DM children in three groups were displayed in Table 1.
3.2. Association between Vitamin D levels and different variables by univariate analysis

The correlations between Vitamin D levels and other anthropometric and biochemical variables in the univariate linear regression analysis were shown in Table 2. Univariate linear regression analysis was performed to determine the relationships between Vitamin D levels and clinical parameters. As shown in Table 2, for the unadjusted model, we did not observe a significant correlation between Vitamin D levels and LDL-C (P=0.634). Other variables that remained significantly associated with Vitamin D levels were sex, age, FPG, and C-peptide (all p<0.05). No significant association was observed between Vitamin D levels and BMI, HbA1c, CRP, PCT, TG, TC, HDL-C, LDL-C, Non-HDL-C, ApoA, ApoB, Lipoprotein, and Free fatty acid (all p>0.05).

3.3. Independent association of Vitamin D levels and LDL-C by piecewise multivariate linear regression

In our study, we analyzed the nonlinear relationship between Vitamin D levels and LDL-C (Fig. 1). As shown in Fig. 1, the scatter plot of the distribution for LDL-C and Vitamin D levels in T1DM children was shown and the smooth curve fitting was performed after adjusting for underlying confounding factors, including age, sex, BMI, FPG, C-peptide. The patient's LDL-C shown a nonlinear relationship with Vitamin D levels and the resulting curve presented a two-stage change and an inflection point. When LDL-C was greater than the inflection point, there was an inverse relationship between LDL-C and Vitamin D levels; however, if the value was less than the inflection point, there was a positive relationship between LDL-C and Vitamin D levels.

Threshold effect analysis for the relationship between LDL-C and Vitamin D levels was shown in Table 3. As shown in Table 3, linear regression and two-piecewise linear regression were performed to fit the association and select the best fit model based on P-value for the Logarithmic likelihood ratio test (LRT test). Because the P-value for the LRT test was less than 0.05, we chose two-piecewise linear regression (Model II) for fitting the association between LDL-C and Vitamin D levels because it can accurately display the relationship. Using two-piecewise linear regression and a recursive algorithm after adjusting for age, sex, BMI, FPG, and C-peptide, we calculated that the turning point was 3.1 mmol/L. A multivariate piecewise linear regression model indicated a significant negative association between LDL-C and Vitamin D levels when LDL-C was greater than 3.1 mmol/L (β -2.9, 95% CI -5.4,-0.5; P=0.022). However, we did not observe a significant relationship between LDL-C and Vitamin D levels when LDL-C was lower than 3.1 mmol/L (β 2.4, 95% CI -0.2,5.1; P=0.076).

4. Discussion

In our study, we concluded that there was a nonlinear relationship between Vitamin D levels and LDL-C in children with T1DM, and the LDL-C turning point was 3.1 mmol/L. The negative relationship between Vitamin D levels and LDL-C was significant only when LDL-C reached the turning point.

To the best of our knowledge, the relationship between Vitamin D levels and LDL-C in patients with T1DM has not been fully evaluated. Metabolomics techniques have shown that patients who progress to
diabetes have different levels of certain lipids when compared with persons who remain non-diabetic[14]. Diabetic patients with dyslipidemia commonly suffer from a higher risk of adverse outcomes[4]. Guidelines have traditionally advocated that LDL-C concentration represents the primary goal for lipid-lowering intervention[4]. In our study, we observed a nonlinear relationship between Vitamin D levels and LDL-C in children with T1DM suggesting that a certain LDL-C level may influence the serum Vitamin D levels. This finding suggests that the deficiency or insufficiency of Vitamin D in T1DM children with high LDL-C levels should be considered.

A link between autoimmune states and vitamin D deficiency or insufficiency has been identified. Vitamin D is an immunomodulatory hormone and the active form of vitamin D[(1,25(OH)_{2}D)] exerts immunologic activities on both innate and adaptive immune systems through VDR as well as endothelial membrane stability[11]. This notion can support our finding that Vitamin D may be a protective factor against elevated LDL-C. Besides, Animal studies have revealed that administration of vitamin D or its metabolites can change the occurrence and development of various immune-related diseases[16, 17]. Mathieu et al. found that long-term treatment with high doses of calcitriol (5 µg/kg/d or alternate days) was able to reduce the incidence of diabetes in non-obese diabetic (NOD) mice without major side effects[18]. The cause and effect relationship between vitamin D deficiency and T1DM has yet to be widely researched and it is still controversial what level of serum Vitamin D level is optimal[17, 19]. Now, researchers are investigating the effect of vitamin D supplementation as adjuvant immunomodulatory therapy for the treatment of T1DM[17]. It is recommended to increase vitamin D intake and appropriate sunlight exposure to maintain serum 25(OH)D at least 30 ng/mL (75 nmol/L) and preferably 40-60 ng/mL (100-150 nmol/L) to achieve the optimal overall health benefits of vitamin D[11]. However, different doses, formulations, and analogs of vitamin D should be studied extensively and continuously.

Besides, our study also observed a negative relationship between age, sex, FPG, and Vitamin D levels. In short, the level of Vitamin D decreases as age increases. Besides, male children with T1DM were prone to be deficient or insufficiency in Vitamin D. This finding was also consistent with the recent cross-sectional study of Israeli that Vitamin D levels decrease in children with T1DM[20].

In our study, we confirmed the relationship between Vitamin D levels and LDL-C in children with T1DM. The level of Vitamin D is related not only to immunomodulatory effects but also to lipid metabolism.

The following limitations of our study should be addressed. Firstly, the findings were restricted to a selected group of Chinese patients from a single center. Hence, results should be interpreted with caution. Secondly, glucose and all lipid parameters measures were evaluated based on a single measurement, variability existed in experimental error, which may cause some bias and attribute to the discrepancies of results. Thirdly, although we did find an association between LDL-C and Vitamin D, other potential factors were not evaluated in the present study, such as dietary characteristics and other concomitant therapies influencing lipid and Vitamin D levels. Moreover, we had a comparatively small sample size of subjects, which had become smaller when dividing into three groups. Therefore, the results and conclusions in our
study should be regarded as preliminary. A larger sample size and multicentre trials are necessary to confirm our findings.

5. Conclusions

In conclusion, our study identified the nonlinear connection between LDL-C and Vitamin D levels in Chinese children with T1DM after adjusting for potential confounders. Above a certain LDL-C level, a powerful relationship between LDL-C and Vitamin D levels existed. This finding suggests that the deficiency or insufficiency of Vitamin D in T1DM children with high LDL-C levels should be considered, especially LDL-C is higher than 3.1 mmol/L, which provides evidence of the timing of Vitamin D supplementation in T1DM children.

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Tables

**TABLE 1** Demographic and clinical characteristics in patients with T1DM divided into 3 groups according to serum Vitamin D level.
### TABLE 2: Association between Vitamin D levels and different variables by univariate analysis

| Variables                  | Deficiency of Vitamin D (n=46) | Insufficiency of Vitamin D (n=35) | Sufficient of Vitamin D (n=62) | P value |
|----------------------------|--------------------------------|-----------------------------------|-------------------------------|---------|
| Boys (%)                   | 28/46(60.9)                    | 13/35(37.1)                       | 19/62(30.6)                   | p=0.006 |
| Girls (%)                  | 18/46(39.1)                    | 22/35(62.9)                       | 43/62(69.4)                   | p=0.236 |
| Age (years)                | 8.26±3.45                      | 7.17±3.42                         | 7.16±3.77                     | p=0.511 |
| Age of onset (years)       | 6.63±3.07                      | 6.29±3.04                         | 5.89±3.60                     | p=0.040 |
| BMI (kg/m²)                | 15.28±1.94                     | 15.24±1.99                        | 16.31±2.31                    | p=0.040 |
| BMI SDS                    | 0.14±0.97                      | 0.12±0.99                         | 0.66±1.15                     | p=0.040 |
| FPG (mmol/L)               | 22.87±10.87                    | 19.40±11.03                       | 17.88±9.56                    | p=0.041 |
| HbA1c (%)                  | 12.75±4.12                     | 12.31±3.57                        | 11.52±3.85                    | p=0.253 |
| C-peptide (nmol/l)         | 0.10±0.95                      | 0.13±0.10                         | 0.17±0.16                     | p=0.040 |
| CRP (mg/L)                 | 4.88±0.66                      | 4.56±0.10                         | 2.37±4.35                     | p=0.271 |
| PCT (ng/ml)                | 0.87±0.33                      | 0.35±0.18                         | 1.81±0.52                     | p=0.545 |
| TG (mmol/L)                | 4.12±4.67                      | 2.90±3.31                         | 2.35±2.82                     | p=0.046 |
| TC (mmol/L)                | 5.31±1.88                      | 4.89±1.00                         | 4.90±1.27                     | p=0.282 |
| HDL-C (mmol/L)             | 1.24±0.48                      | 1.42±0.53                         | 1.43±0.56                     | p=0.143 |
| LDL-C (mmol/L)             | 3.00±1.56                      | 2.75±0.96                         | 2.84±0.91                     | p=0.634 |
| Non-HDL-C (mmol/L)         | 4.10±1.99                      | 3.48±1.18                         | 3.49±1.45                     | p=0.109 |
| Apo A (mmol/L)             | 134.00±29.63                   | 140.35±25.39                      | 135.30±30.61                  | p=0.607 |
| Apo B (mmol/L)             | 109.84±44.93                   | 100.18±28.57                      | 103.10±32.29                  | p=0.463 |
| Lipoprotein (mmol/L)       | 26.20±7.29                     | 20.80±3.32                        | 21.04±5.37                    | p=0.757 |
| Free fatty acid (mmol/L)   | 5.20±4.98                      | 0.97±0.46                         | 1.00±0.57                     | p=0.335 |

**Abbreviations:** BMI: body mass index; BMI SDS: body mass index standard deviation scores; FPG: fasting plasma glucose; HbA1c: hemoglobin A1c; CRP: C reactive protein; TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; Apo A: apolipoprotein A; Apo B: apolipoprotein B.
| Characteristics          | β/OR (95% CI) | P value |
|--------------------------|---------------|---------|
| **Boys (%)**             | 0             |         |
| **Girls (%)**            | 2.9 (0.0, 5.8) | 0.049   |
| Age(years)               | -0.5 (-0.9, -0.1) | 0.008   |
| BMI (kg/m2)              | 0.6 (-0.2, 1.3) | 0.153   |
| FPG (mmol/l)             | -0.2 (-0.3, -0.0) | 0.024   |
| HbA1c (%)                | -0.4 (-0.9, 0.0) | 0.072   |
| C-peptide(nmol/l)        | 17.3 (6.9, 27.7) | 0.001   |
| CRP (mg/L)               | -0.1 (-0.3, 0.0) | 0.102   |
| PCT (ng/ml)              | -0.0 (-0.2, 0.2) | 0.865   |
| TG (mmol/L)              | -0.3 (-0.7, 0.1) | 0.092   |
| TC (mmol/L)              | -1.0 (-2.0, 0.0) | 0.063   |
| HDL-C (mmol/L)           | 0.4 (-2.2, 2.9) | 0.789   |
| LDL-C (mmol/L)           | -0.7 (-1.9, 0.5) | 0.253   |
| Non-HDL-C (mmol/L)       | -0.9 (-1.8, 0.1) | 0.069   |
| Apo A (mmol/L)           | -0.0 (-0.1, 0.0) | 0.533   |
| Apo B (mmol/L)           | -0.0 (-0.1, 0.0) | 0.279   |
| Lipoprotein (mmol/L)     | -0.0 (-0.1, 0.0) | 0.310   |
| Free fatty acid (mmol/L) | -0.0 (-0.2, 0.1) | 0.534   |

Abbreviations: BMI: body mass index; BMI SDS: body mass index standard deviation scores; FPG: fasting plasma glucose; HbA1c: hemoglobin A1c; CRP: C reactive protein; TG: triglyceride; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; Apo A: apolipoprotein A; Apo B: apolipoprotein B.

### TABLE 3
Threshold effect analysis for the relationship between LDL-C and Vitamin D levels

| Models         | Vitamin D Crude β (95% CI) | p-value | Adjusted β (95% CI) | p-value |
|----------------|----------------------------|---------|---------------------|---------|
| Model I        |                            |         |                     |         |
| One line slope | -0.7 (-1.9, 0.5)           | 0.253   | -0.4 (-1.7, 1.0)    | 0.584   |
| Model II       |                            |         |                     |         |
| Turning point  | 3.2 (3.1)                  |         |                     |         |
| < 3.2 slope 1  | 1.6 (-0.3, 3.5)            | 0.095   | 2.4 (-0.2, 5.1)     | 0.076   |
| > 3.2 slope 2  | -3.9 (-6.2, -1.5)          | 0.002   | -2.9 (-5.4, -0.5)   | 0.022   |
| LRT test       | 0.003                      |         | 0.009               |         |

Model I, linear analysis; Model II, non-linear analysis. LRT test, Logarithmic likelihood ratio test. (p value < 0.05 means Model II is significantly different from Model I, which indicates a non-linear relationship); Crude: no adjustment; Adjusted: adjusted for age, sex, BMI, FPG, C-peptide. BMI: body mass index; FPG: fasting plasma glucose.

**Figures**
Figure 1

The relationship between LDL-C and Vitamin D levels was determined by smooth curve fitting. The scatter plot of the distribution for LDL-C and Vitamin D levels in T1DM children (A) and the curve fitting line for LDL-C and Vitamin D levels in T1DM children (B) are shown. Adjusted variables: age, sex, BMI, FPG, C-peptide. BMI: body mass index; FPG: fasting plasma glucose; T1DM: type 1 diabetes mellitus.