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Serum vitamin B12 levels and glycemic fluctuation in patients with type 2 diabetes mellitus

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Abstract

Purpose: The aim of the study was to explore the correlation between serum vitamin B12 levels and glycemic fluctuation in patients with type 2 diabetes mellitus (T2DM).

Methods: This study included 202 T2DM patients in whom blood glucose levels were recorded using a continuous glucose monitoring system retrospectively. Glycemic fluctuation was determined using the average daily risk range (ADRR), a diabetes-specific measure of the risk for hyper- and hypoglycemia.

Results: Serum vitamin B12 levels were higher in T2DM patients with wider glycemic fluctuations than in those with minor glycemic fluctuations ($p < 0.001$). We observed a positive correlation between serum vitamin B12 levels and ADRR in both T2DM patients who received and did not receive metformin therapy ($r = 0.388$, $p < 0.001$ and $r = 0.280$, $p = 0.004$, respectively). Multiple linear regression analysis showed that serum vitamin B12 levels were independently correlated with ADRR in T2DM patients who received and did not receive metformin therapy (beta = 0.367, $p < 0.001$ and beta = 0.410, $p < 0.001$, respectively).

Conclusions: Serum vitamin B12 levels are correlated with glycemic fluctuation in patients with T2DM and may serve as an underlying useful biomarker of glycemic fluctuation in T2DM patients, treated with or without metformin therapy.

Keywords: average daily risk range, glycemic fluctuation, serum vitamin B12, type 2 diabetes mellitus

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Introduction

Type 2 diabetes mellitus (T2DM) is a common chronic disease associated with several complications that negatively affect patients' quality of life and contribute to a significant clinical and economic burden to society.[1] It is well known that optimal blood glucose control is important to reduce the incidence of diabetes complications, and extreme glycemic fluctuation is an independent risk factor for atherosclerosis in patients with T2DM.[2,3] Moreover, evaluation of daily glucose fluctuations provides baseline information for the clinical management of T2DM.[4] Therefore, regular glycemic fluctuation monitoring should be considered an important component of routine clinical management of patients with T2DM.

Reportedly, B-vitamins, particularly vitamin B12, are implicated in the pathogenesis of glucose intolerance, and vitamin B12 levels tend to decrease with increasing severity of glucose tolerance.[5] It is well known that vitamin B12 deficiency is associated with increased risk of gestational DM.[6] A recent study also reported that add-on supplementation with vitamin B12 can improve glycemic control and insulin resistance in T2DM patients.[7] Reportedly, patients with diabetes have higher serum vitamin B12 levels than those with normal glycemic control, and fructosamine levels are significantly correlated with serum vitamin B12 levels in patients with DM,[8] which suggests that serum vitamin B12 levels may be associated with glycemic control in T2DM. However, no study has investigated the correlation between

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serum vitamin B12 levels and glycemic fluctuation in patients with T2DM. In this study, we investigated the correlation between serum vitamin B12 levels and glycemic fluctuation in T2DM patients.

Patients and methods

Patients

We retrospectively investigated 202 T2DM patients who visited the Suzhou Hospital of Anhui Medical University. All patients underwent laboratory tests for fasting glucose measurement, and T2DM was diagnosed based on the American Diabetes Association criteria.[9] Patients with cardiovascular disease, cerebrovascular disease, active infection, pregnancy, or malignant tumors were excluded from the study. The study was performed in accordance with the Declaration of Helsinki, and the study protocol was approved by the Ethics Committee of Suzhou Hospital of Anhui Medical University (2020031) with a waiver of informed consent.

Continuous blood glucose monitoring

Blood glucose monitoring was performed using the continuous glucose monitoring system (CGMS) in all patients. Briefly, the probe was placed under the abdominal skin, and dynamic blood glucose parameters were measured over 72 h using the Medtronic software. Patients were instructed to avoid intense exercise and bathing while they wore the CGMS. The average daily risk range (ADRR), a reliable measure computed from routine self-monitored blood glucose variability data, was used to determine glycemic fluctuation in patients with T2DM.[10]

Statistical analysis

Continuous variables are expressed as median (interquartile range) and categorical variables as numbers. Categorical variables were compared using the chi-square test. Statistical significance was analyzed using the Mann–Whitney U test for continuous variables, and bivariate correlation between two continuous variables was analyzed using the Spearman correlation test. Potentially factors associated with vitamin B12 levels were considered as independent variables, then, age, sex, body mass index (BMI), glycated hemoglobin, the levels of blood glucose 0 h, blood glucose 2 h, C-peptide 0 h, C-peptide 2 h, and ADRR were included as independent variables for multiple linear regression analysis. Stepwise multiple linear regression analysis was used to determine the correlation between serum vitamin B12 levels and ADRR in the different groups. All statistical analyses were performed using the SPSS software, version 25.0. A p value of <0.05 was considered statistically significant.

Results

Comparison of baseline data based on the median ADRR in T2DM patients

Patients were categorized into two groups based on the median ADRR for intergroup comparison of baseline data. We observed significant differences in sex, metformin therapy, BMI, glycated hemoglobin, glucose 0 h, glucose 2 h, C-peptide 0 h, C-peptide 2 h, serum alanine transaminase, and aspartate transaminase. Serum vitamin B12 levels were significantly higher in T2DM patients who showed wider glycemic fluctuations than in those with minor glycemic fluctuations ($p<0.001$) (Table 1).

Intergroup comparison of correlation between serum vitamin B12 levels and ADRR

A positive correlation was observed between serum vitamin B12 levels and ADRR ($r=0.346$, $p<0.001$) in patients with T2DM. To exclude the effects of metformin therapy on vitamin B12 levels, we further analyzed the correlations between serum vitamin B12 levels and ADRR in T2DM patients who were and were not administered metformin therapy. Results showed that serum vitamin B12 levels were positively correlated with ADRR in both groups of T2DM patients (those who were and were not administered metformin therapy) ($r=0.388$, $p<0.001$ and $r=0.280$, $p=0.004$, respectively) (Table 2).

Intergroup comparison of correlation between serum vitamin B12 and glucose metabolic parameters

Serum vitamin B12 levels were positively correlated with glycated hemoglobin ($r=0.142$, $p=0.044$) and glucose 2 h ($r=0.271$, $p<0.001$) and negatively correlated with C-peptide 0 h ($r=-0.284$, $p<0.001$) and C-peptide 2 h ($r=-0.280$, $p<0.001$) in patients with T2DM. Furthermore, serum vitamin B12 levels were

Table 1. Comparison of baseline data based on the median ADRR in T2DM patients.

	Group I	Group II	*p* value
Sex			
Male	75	61	0.036
Female	26	40	
Age (years)	55 (48–63)	60 (47–67)	0.076
Metformin therapy			
Yes	59	38	0.003
No	42	63	
Body mass index (kg/m^2)	26.3 (23.6–29.1)	24.2 (22.3–26.2)	0.001
Glycated hemoglobin (%)	7.7 (6.7–9.2)	9.4 (8.4–11.5)	<0.001
Glucose 0 h (mmol/l)	6.9 (5.5–8.6)	8.2 (6.8–10.3)	<0.001
Glucose 2 h (mmol/l)	14.4 (11.9–16.1)	17.8 (15.4–20.2)	<0.001
C-peptide 0 h (ng/ml)	1.6 (1.0–2.6)	0.9 (0.5–1.4)	<0.001
C-peptide 2 h (ng/ml)	5.3 (3.3–7.2)	2.7 (1.7–4.2)	<0.001
Alanine transaminase (U/l)	19 (13.0–33.5)	15.0 (10.0–23.5)	0.004
Aspartate transaminase (U/l)	18.0 (15.0–25.0)	17.0 (13.0–21.5)	0.041
Vitamin B12 (pg/ml)	510.0 (356.0–708.0)	746.0 (496.5–1000.0)	<0.001

ADRR, average daily risk range; Group I, less than median ADRR; Group II, greater than median ADRR; T2DM, type 2 diabetes mellitus.

positively correlated with glycated hemoglobin ($r=0.319$, $p=0.001$) and glucose 2 h ($r=0.291$, $p=0.004$) and negatively correlated with C-peptide 0 h ($r=-0.234$, $p=0.021$) and C-peptide 2 h ($r=-0.286$, $p=0.005$) in T2DM patients who received metformin therapy. We observed a positive correlation between serum vitamin B12 and glucose 2 h levels ($r=0.252$, $p=0.010$) in T2DM patients who did not receive metformin therapy, and serum vitamin B12 levels were negatively correlated with C-peptide 0 h ($r=-0.304$, $p=0.002$) and C-peptide 2 h levels ($r=-0.246$, $p=0.011$) in T2DM patients who did not receive metformin therapy (Table 2).

Multiple linear regression analysis of serum vitamin B12 levels and ADRR across different study groups

Multiple linear regression analysis was used to determine the correlations between serum vitamin B12 levels and ADRR in the different groups. The serum vitamin B12 level was defined as a dependent variable for multiple linear regression analysis. Results revealed that serum vitamin B12 levels were independently correlated with ADRR (beta = 0.326, $p<0.001$) in all T2DM patients, in T2DM patients who received metformin therapy (beta = 0.367, $p<0.001$), as well as in T2DM patients who did not receive metformin therapy (beta = 0.410, $p<0.001$) (Table 3).

Discussion

Metformin is recognized as an important component of antidiabetic (T2DM) therapy, in recent years.[11] However, metformin use is shown to be associated with the risk of vitamin B12 deficiency in patients with T2DM.[12] The high prevalence of vitamin B12 deficiency in patients with T2DM who receive metformin treatment may be

Table 2. Correlations between serum vitamin 12 and parameters in different groups.

	All T2DM patients		T2DM patients with metformin therapy		T2DM patients without metformin therapy	
	r	*p* value	*r*	*p* value	*r*	*p* value
Age	0.041	0.559	0.061	0.551	−0.005	0.962
Body mass index	−0.115	0.102	−0.101	0.327	−0.076	0.444
Glycated hemoglobin	0.142	0.044	0.319	0.001	0.018	0.858
Glucose 0 h	0.097	0.171	0.165	0.105	0.062	0.527
Glucose 2 h	0.271	<0.001	0.291	0.004	0.252	0.010
C-peptide 0 h	−0.284	<0.001	−0.234	0.021	−0.304	0.002
C-peptide 2 h	−0.280	<0.001	−0.286	0.005	−0.246	0.011
Alanine transaminase	−0.078	0.267	−0.050	0.625	−0.060	0.544
Aspartate transaminase	0.008	0.905	−0.028	0.783	0.082	0.406
ADRR	0.346	<0.001	0.388	<0.001	0.280	0.004

ADRR, average daily risk range; T2DM, type 2 diabetes mellitus.

Table 3. Multiple linear regression analysis of serum vitamin B12 levels and ADRR across different study groups.

	Unstandardized coefficients		Standardized coefficients	*t*	*p* value
	B	Standard error	Beta		
All T2DM patients					
ADRR	6.234	1.279	0.326	4.874	<0.001
T2DM patients with metformin therapy					
ADRR	7.167	1.863	0.367	3.847	<0.001
T2DM patients without metformin therapy					
Glycated hemoglobin	−25.863	11.457	−0.257	−2.257	0.026
ADRR	7.909	2.192	0.410	3.608	<0.001

ADRR, average daily risk range; T2DM, type 2 diabetes mellitus.

attributable to metformin-induced inhibition of calcium-dependent absorption of the intrinsic factor–vitamin B12 complex in the ileum.[13,14] Therefore, vitamin B12 supplementation is considered an integral component of the routine treatment regimen for T2DM patients, particularly for those who receive prolonged metformin treatment.[12,15] We investigated the correlation between serum vitamin B12 levels and ADRR in T2DM patients and observed a positive correlation between serum vitamin B12 levels and ADRR in these patients, both those who were and were not

treated with metformin, which suggests that serum vitamin B12 levels may be associated with glycemic fluctuation in T2DM patients, regardless of metformin therapy.

Vitamin B12, also referred to as cobalamin, is an essential micronutrient predominantly derived from animal sources. Transcobalamin II (TCB II) is a cobalamin transport protein; cobalamin absorbed from the gastrointestinal tract is delivered into the circulation via the portal system. Cobalamin transferred from peripheral blood to cells for utilization results in formation of the combined TCB II–cobalamin complex in the circulation, which is recognized by the TCB II receptor on cell membranes for utilization.[16] Therefore, TCB II plays an important role in vitamin B12 utilization. Vitamin B12 oversupplementation was shown to increase TCB expression owing to overproduction or reduced clearance, and a quantitative deficiency or reduced affinity of TCB can lead to increased serum vitamin B12 levels.[17] In fact, serum TCB may undergo glycosylation in diabetic patients, with consequently increased serum vitamin B12 levels.[8] In addition, vitamin B12 is 'trapped' in the plasma in patients with hyperglycemia, which leads to increased serum vitamin B12 levels, which explains the positive correlation with higher serum glycated hemoglobin levels.[18,19] Our results showed a positive correlation between serum vitamin B12 levels and glycated hemoglobin in T2DM patients who did not receive metformin therapy, which is consistent with the findings reported by the aforementioned studies. Thus, we speculate that hyperglycemia may inhibit vitamin B12 utilization and consequently increase serum vitamin B12 levels in patients with T2DM. Moreover, hyperglycemia may interfere with vitamin B12 metabolism; poor glycemic control increases the accumulation of advanced glycation end products, which can cause glycation of the vitamin B12 receptor and overwhelm it and reduce its function. Furthermore, owing to its nonenzymatic glycation, vitamin B12 is not recognized by the receptor during cellular uptake. These factors prevent vitamin B12 entry into and utilization by cells and consequently increase serum vitamin B12 levels in patients with T2DM.[19]

Studies have reported that free vitamin B12 can be absorbed passively without utilizing the intrinsic factor and cubam;[20] therefore, exogenous vitamin B12 supplementation may lead to absorption of vitamin B12 via a passive pathway and increase serum B12 levels. However, long-term vitamin B12 administration can trigger production of anti-TCB II antibodies and reduce TCB II clearance,[21,22] which impairs intracellular utilization of vitamin B12 and aggravates intracellular vitamin B12 deficiency, even high vitamin B12 levels in peripheral circulation, which may affect cellular glucose metabolism and homeostasis in patients with T2DM.

Following are the limitations of this study: (a) the small sample size is a drawback, and further large-scale studies are warranted to confirm our results. (b) Dietary patterns were not assessed in this study; variations in dietary patterns may have affected vitamin B12 intake in patients with T2DM. (c) Vitamin B12 or mecobalamin use was not evaluated in T2DM patients, which may potentially affect the conclusions drawn in this study. (d) Causality between serum vitamin B12 levels and glycemic fluctuation was not confirmed owing to the study's cross-sectional design.

Conclusions

This study highlights the correlation between serum vitamin B12 levels and glycemic fluctuation in patients with T2DM and that serum vitamin B12 may be a potentially useful indicator of glycemic fluctuation in T2DM patients, regardless of metformin therapy.

Author contribution(s)

Wei Li: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing – original draft.

Jing Zhao: Investigation.

Ling-Ling Zhu: Investigation.

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Conflict of interest statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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