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Randomized Control Trials

Vitamin B complex supplementation as a homocysteine-lowering therapy for early stage diabetic nephropathy in pediatric patients with type 1 diabetes: A randomized controlled trial

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SUMMARY

Background: Homocysteine levels are elevated in patients with type 1 diabetes mellitus (T1DM) and could induce renal injury. B vitamins have an important role in preventing microvascular complications of diabetes.
Aim: We performed a randomized-controlled trial of oral supplementation with vitamin B complex as an adjuvant therapy for nephropathy in pediatric T1DM patients and assessed its relation to homocysteine and cystatin C as a marker of nephropathy.
Methods: This trial included 80 T1DM patients with microalbuminuria, despite oral angiotensin-converting enzyme inhibitors, aged 12–18 years with at least 5 years disease duration and HbA1c ≤8.5%. Patients were randomly assigned into two groups; intervention group which received oral vitamin B complex (B1, B6 and B12) once daily and placebo group. Both groups were followed-up for 12 weeks with assessment of plasma homocysteine, HbA1c, urinary albumin excretion (UAE) and cystatin C.
Results: Both groups were well-matched in baseline clinical and laboratory parameters. Baseline homocysteine levels were elevated in both groups compared with reference control values. After 12 weeks, supplementation with vitamin B complex for the intervention group resulted in a significant decrease of homocysteine, fasting blood glucose, HbA1c, triglycerides, total cholesterol, UAE and cystatin C compared with baseline levels ($p < 0.001$) and with placebo group ($p < 0.001$). No adverse reactions were reported. Baseline cystatin C was negatively correlated to vitamin B12 ($r = -0.77$, $p = 0.001$).
Conclusions: Vitamin B complex improved glycemic control and renal function through decreasing homocysteine and could be a safe and effective strategy for treatment of early stage nephropathy in pediatric T1DM. This trial was registered at ClinicalTrials.gov (NCT03594240).

1. Introduction

Diabetic nephropathy (DN), a major microvascular complication of both type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM), is an important cause of end stage renal disease (ESRD) in the developed countries [1]. The initial stage of development of nephropathy, incipient nephropathy, is characterized by the onset of persistent microalbuminuria and hyperfiltration [2].

Human cystatin C is a low molecular weight cysteine protease inhibitor produced by almost all nucleated cells present in the body. It is filtered freely in the glomerulus, gets reabsorbed in the proximal tubules and degraded [3]. Cystatin C is mainly used as a biomarker of kidney function and can be used to screen patients with poorly controlled DM or hypertension when serum creatinine level is inconclusive [4]. Cystatin C correlates closely to glomerular filtration rate (GFR) in children [5].

Although with established conventional therapy for glycemic and blood pressure control in patients with DN, and the use of inhibitors of the renin–angiotensin–aldosterone system (RAAS), a significant proportion of diabetic patients develop chronic kidney

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disease (CKD) and progress to ESRD, indicating a need for additional treatments [6].

Thiamine (B1), Riboflavin, Niacin (B3), Panthotenic acid, Pyridoxine (B6), Biotin, Cobalamin (B12) and Folic acid are usually grouped as B-vitamins, and most of them have been linked to T2DM [7]. Vitamin B12 is an essential micronutrient required for optimal hemopoietic, neuro-cognitive and cardiovascular function. Biochemical and clinical vitamin B12 deficiency has been demonstrated to be highly prevalent among adult patients with T1DM and T2DM [8,9]. Those patients showed similarly decreased plasma thiamine concentrations [10,11]. Moreover, data on vitamin B6 deficiency in T2DM patients with nephropathy have been documented [12].

Among patients with T1DM, there are no clear guidelines regarding screening for vitamin B12 deficiency. However, due to the high prevalence of pernicious anemia and subsequent vitamin B12 deficiency among T1DM patients, it would be pragmatic to screen at diagnosis and then later yearly for 3 years, then five yearly thereafter or in presence of any clinical indication since vitamin B12 deficiency can develop at any time [9].

Homocysteine levels are elevated in T1DM patients with DN and proliferative retinopathy [13–16]. Animal studies showed that Tri-B (B1, B6 and B12) treatment effectively improved the changes in malondialdehyde, lipid profile, blood glucose level and insulin [17]. B-vitamin therapy has been shown to lower the plasma concentration of homocysteine and improve endothelial function [18,19]. Studies on vitamin B6 and high-dose vitamin B1 suggested inhibition of albuminuria in diabetic animal models [20,21].

To the best of our knowledge, no previous study assessed the role of vitamin B complex in diabetes associated complications in particular DN among pediatric patients with T1DM and there is insufficient evidence to recommend its supplementation for those patients. Therefore, this study was undertaken to investigate the role of vitamin B complex (B1, B6 and B12) as an adjuvant therapy for DN in children and adolescents with T1DM and assess its relation to homocysteine level, glycemic control, microalbuminuria and cystatin C as a marker of nephropathy.

2. Materials and methods

This prospective randomized, double-blinded and placebo-controlled trial was approved from the local ethical committee and registered in the ClinicalTrials.gov (NCT03594240). Eighty type 1 diabetic patients with nephropathy who met the inclusion criteria were recruited from the regular attendants of the Pediatric Diabetes Clinic, Pediatric Hospital, Ain Shams University. The study protocol was approved by the Ethical Committee of Ain Shams University, and an informed consent was obtained from each patient or their legal guardians before participation. Reporting of the study conforms to Consolidated Standards of Reporting Trials 2010 statement [22].

Patients were defined according to the criteria of American Diabetes Association [23]. Inclusion criteria were patients with T1DM on insulin therapy, aged 12–18 years with at least 5 years disease duration, active diabetic nephropathy in the form of microalbuminuria (urinary albumin excretion [UAE] 30–299 mg/g creatinine), and hemoglobin A1c (HbA1c) ≤8.5% (69 mmol/mol). The presence of persistent microalbuminuria was confirmed by finding two or all of three samples abnormal over a 3- to 6-months period prior to study despite angiotensin converting enzyme inhibitors (ACE-Is) [24–26].

Exclusion criteria included patients with any clinical evidence of infection, renal impairment due to causes other than diabetes, other diabetic complications than nephropathy, elevated liver enzymes, hyper- or hypo-thyroidism, hypertension, neoplasm, taking any vitamins or food supplements one month before study and participation in a previous investigational drug study within the three months preceding screening. All participants were asked to refrain from substantial changes in their lifestyle habits in the course of the study.

2.1. Sample size

Sample size was calculated using PASS® version 11 program, setting the type-1 error (α) at 0.05 and the power (1-β) at 0.8. According to literature, assuming that the cystatin C level in treatment group is 558.7 ± 140.3 after treatment while it is assumed to be 676.8 ± 159.8 for placebo group, calculation according to these values produced a sample size of 40 cases in each group, after taking in consideration a 10% drop out rate. The analyses were conducted based on intention-to-treat (ITT) principle. In cases of drop-out, data were not replaced.

2.2. Randomization and study groups

A total of 146 patients with diabetic nephropathy were screened for eligibility; 32 patients did not meet inclusion criteria, 9 patients declined to participate, 25 patients were excluded and 80 patients were enrolled (Fig. 1). Drug administration was according to a predetermined schedule generated from random numbers in a 1:1 manner based on a computer-generated randomization sequence maintained within the investigational drug pharmacy with allocation concealment by opaque sequentially numbered sealed envelope. The 80 patients were randomly assigned into 2 groups (40 patients in each group) to receive either vitamin B complex, or matching placebo. Patients in both groups received oral ACE-Is captopril 25 mg tablet (manufactured by SmithKline Beecham-Egypt L.L.C. an affiliated company to GlaxoSmithKline) provided that their blood pressure could be maintained within normal range for age. Vitamin B group included pediatric patients with diabetic nephropathy receiving oral vitamin B complex tablets (Neurorubine TM – Forte Lactab TM Mepha Pharma Egypt S.A.E manufactured by Medical Union Pharmaceuticals) in a dose of one lactab

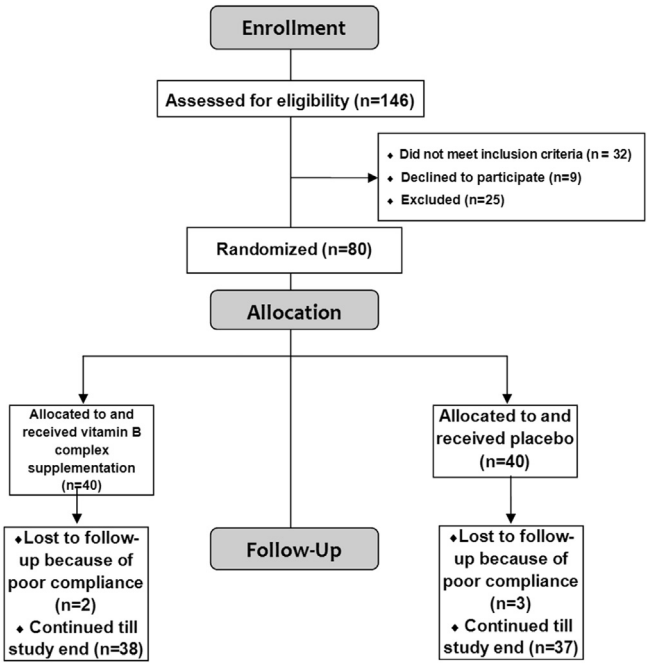

Fig. 1. CONSORT flow diagram for the enrolled patients with diabetic nephropathy.

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tablet once daily for three months. The Neurorubine – Forte lactab contains 200 mg of Thiamine Nitrate (vitamin B1), 50 mg Pyridoxine hydrochloride (vitamin B6) and 1000 μg Cyanocobalamine (vitamin B12).

2.3. Dietary intake

Simultaneously with pharmacological treatment, nutrient intake was tabulated from 24 -hour dietary recall, which was carried out by a specialist pediatric dietician through interview with the mother or directly with an older child. The advised caloricity of the diet varied from 1200 to 2400 kcal per day with respect to patient's age and gender. All patients were instructed to follow the hospital regular balanced diet schedule with optimal macronutrient distribution to prevent overload of carbohydrate and fat consumption and to sustain ideal body weight and optimal growth. The carbohydrate content of the meals was 50–55% of the total daily calories. Carbohydrate counting was used. The protein content of the meals was 15–20% of total energy intake and the fat intake was <35% of the total caloric intake mainly monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) [27,28]. All participants were asked to refrain from substantial changes in their lifestyle habits in the course of the study and they were counseled to exclude taking any vitamins or food supplements one month before study entry.

2.4. Baseline clinical assessment

The studied patients were subjected to detailed medical history and thorough clinical examination with special emphasis on age of onset of diabetes, insulin therapy and chronic diabetic complications (nephropathy, peripheral neuropathy, retinopathy or cardiovascular ischemic events), dietary intake and adverse effects during administration of treatment. Anthropometric measurements and blood pressure were recorded.

2.5. Sample collection and laboratory analysis

Peripheral blood samples were collected on potassium-ethylene diamine tetra-acetic acid (K2-EDTA) in sterile vacutainer tubes (final concentration of 1.5 mg/mL) (Beckton Dickinson, Franklin Lakes, NJ, USA) for assessment of HbA1c. For biochemical analysis, clotted blood samples were obtained, and serum was separated by centrifugation for 15 min at 1000×. Serum was stored at −80 °C till subsequent use. Blood was collected at the start of the study (day 0) and at 12 weeks.

Fasting blood glucose (FBG) levels, fasting lipid profile and serum creatinine were measured using Cobas Integra 800 (Roche Diagnostics, Mannheim, Germany). Assessment of mean HbA1c% in the year preceding the study was performed using D-10 (BioRad, Marnes La Coquette, France). Estimated GFR (eGFR) was calculated using the Cockgroft-Gault equation [29] as the analysis was done for patients over 12 years of age [30]. UAE was measured for all the enrolled patients with microalbuminuria at the beginning of study in an early morning urine sample (at 8 am) as urinary albumin-to-creatinine ratio (UACR) by an immuno-nephelometric method. Determination of serum levels of vitamin B12 was done using Cobas e 411 (Roche Diagnostics, Mannheim, Germany). Vitamin B1, B6, cystatin C and serum homocysteine were assessed by enzyme linked immunosorbent assay (ELISA). The kits were supplied by Aviva Systems Biology, Corp, San Diego, CA, USA for vitamin B1 and My Biosource, Corp, San Diego, CA, USA for vitamin B6. Serum cystatin C was measured using kits from SinoGeneClon Biotech Co., Ltd, Hangzhou, China while serum homocysteine levels were assessed by IBL International GmbH, Hamburg, Germany; REF AX51301.

2.6. Follow up and endpoints

Planned duration of the treatment with vitamin B was 12 weeks as previously reported [2,31,32] to demonstrate the maximum and clear effect of vitamin B supplementation as an adjuvant therapy on UACR and cystatin C. All patients were closely and clinically followed-up every four weeks during the study period with assessment of FBG and renal functions for evaluating the effects and compliance to both ACE-I and vitamin B complex and for monitoring signs of any potential adverse effect. At the end of the 12 weeks, UACR and cystatin C were also evaluated. Treatment compliance was assessed based on capsule count of dispensed and returned medication and non-compliance was considered if <80% of the study medication or ACE inhibitors had been taken [33].

The primary study endpoint was the change in cystatin C level after the 12 weeks of treatment among diabetic patients receiving vitamin B complex when compared with the placebo group. Secondary outcome measures include UAE and glycemic control compared between intervention and placebo group at 12 weeks. The safety endpoints were the occurrence of any adverse events during study period.

2.7. Statistical analysis

Data were collected, revised, coded and entered to the Statistical Package for Social Science (IBM SPSS) version 20. Kolmogorov–Smirnov test was used to examine the normal distribution of variables. Variables with normal distribution were presented as means and SD. Variables with skewed distribution were presented as median and interquartile range (IQR; 75th and 25th percentiles). To detect differences between the intervention and placebo groups as regards age, disease duration and percent change of the studied variables, we used independent sample Student's t test for quantitative parametric data while data with non-parametric distribution were analyzed using Mann-Whitey test. For comparison of categorical variables, the chi-square test was used. To determine the effects of Vitamin B supplementation on anthropometric measures, blood pressure, insulin dose and biochemical markers, Analysis of Covariance (ANCOVA) was performed to compare mean values between groups adjusted for differences in baseline measures of age, BMI and biochemical indicators. Variables which were not normally distributed, were log-transformed before entering the analysis. To identify within-group changes (before and after 12 weeks of intervention), we applied paired-samples t tests for quantitative parametric data and Wilcoxon rank-sum test for data with non-parametric distribution. Pearson correlation coefficients and multivariable linear regression analysis were employed to assess the parameters affecting cystatin C level. The confidence interval was set to 95% and the margin of error accepted was set to 5%. A p value < 0.05 was considered significant in all analyses.

3. Results

3.1. Baseline clinical and laboratory characteristics of the studied population

Eighty T1DM patients were enrolled; 36 males and 44 females with mean age 15.4 ± 1.6 years (range, 12–18 years). All included patients in both groups had microalbuminuria for at least 6 months prior to the study. Patients were randomly assigned to be 40 in each of the intervention and placebo group. Baseline clinical and laboratory characteristics were similar between both groups (Table 1) and none of patients was hypertensive or obese. All enrolled patients were vitamin B deficient or had low normal levels. Baseline homocysteine levels were elevated in both groups compared with reference control values reported by Papandreou et al. [34].

Table 1
Clinical and laboratory data among type 1 diabetic patients with nephropathy receiving vitamin B complex supplementation and placebo group at baseline and at 12 weeks.

Variable	Vitamin B complex				Placebo				p-value[a]
	Baseline (n = 40)	At 12 weeks (n = 38)	Change	p-value[b]	Baseline (n = 40)	At 12 weeks (n = 37)	Change	p-value[b]	
Age (years)	15.3 ± 1.6	–	–	–	15.5 ± 1.7	–	–	–	0.588*
Males, n (%)	19 (47.5)	–	–	–	17 (42.5)	–	–	–	0.208†
Disease duration (years)	8.4 ± 2.4				8.9 ± 3	–	–	–	0.412*
Weight SDS	−0.96 (−2.17–0.28)	−0.62 (−2.12–0.17)	0.1 (−40.1–2.4)	0.058	−0.78 (−1.48–0.38)	−0.51 (−0.82–0.31)	0.31 (−48.4–1.8)	0.053	0.618
Height SDS	−2.32 (−3.83–0.72)	−1.18 (−3.48–0.57)	3.5 (−22.9–5.1)	0.071	−1.81 (−2.55–0.17)	−1.21 (−2.63–0.49)	2.0 (−38.6–6.1)	0.106	0.826
BMI SDS	0.27 (−0.11–0.77)	0.35 (0.01–0.91)	14 (−38–19.5)	0.196	0.38 (−0.25–1.29)	0.57 (0.09–1.25)	12 (−46–18)	0.092	0.152
Systolic BP SDS	0.22 (−0.8–1.06)	−0.29 (−0.60–0.60)	−56.7 (−92.6–7.3)	0.003	0.37 (−0.44–0.89)	0.52 (−0.11–1.11)	6.1 (−17.9–27.1)	0.076	<0.001
Diastolic BP SDS	0.47 (−0.08–1.51)	−0.01 (−0.44–0.75)	−39 (−101–−4)	<0.001	0.51 (−0.15–1.31)	0.80 (0.44–1.37)	2 (−10–6)	0.056	<0.001
Insulin dose (IU/Kg/day)	1.17 ± 0.2	1.13 ± 0.2	−2.8 (−4.2–1.0)	0.312	1.16 ± 0.3	1.19 ± 0.2	1.0 (−3.2–2.2)	0.526	0.327
FBG (mg/dL)	116.2 ± 25.2	107.7 ± 14.1	−3.2 (−9.1–1.8)	0.005	112.8 ± 18.4	116.4 ± 17	3.82 (−7.3–11.1)	0.131	0.006
RBS (mg/dl)	220.4 ± 50.7	167 ± 37.2	−19 (−28–8)	<0.001	179 ± 32.6	188.5 ± 40.8	3 (−22–19)	0.208	<0.001
Triglycerides (mg/dL)	126.5 ± 22.6	110.4 ± 18.9	−55 (−70–2)	<0.001	136 ± 31	129.6 ± 16.8	−8 (−40–2.1)	0.165	0.034
Total Cholesterol (mg/dL)	189.2 ± 21.8	162.4 ± 25.8	−17.4 (−32.6–5.8)	0.003	208.3 ± 35.9	204.2 ± 20.4	2.43 (−2.27–3)	0.723	<0.001
HDL cholesterol (mg/dL)	48.1 ± 3.3	58.8 ± 1.6	5 (−15–14)	0.037	49.0 ± 4	48.2 ± 3.1	−27 (−45–3)	0.139	0.004
LDL cholesterol (mg/dL)	80.3 ± 12.7	63.2 ± 9.3	−29.2 (−40.8 −18.6)	<0.001	85.6 ± 11.6	83.4 ± 10.2	−3.1 (−5.1–2.7)	0.341	0.01
HbA1c (%)	8.1 ± 0.4	7.5 ± 0.6	−7 (−11–2)	<0.001	7.8 ± 0.6	8.0 ± 0.6	1.1 (−8–6)	0.092	<0.001
HbA1c (mmol/mol)	66.2 ± 8.7	55.4 ± 6.8	−16.7 (−29.8–−9.8)	0.003	64.2 ± 8.1	65.7 ± 7.2	2.6 (−1.1–3.4)		<0.001
Serum creatinine (mg/dL)	0.71 ± 0.12	0.69 ± 0.1	−0.2 (−36–0.2)	0.166	0.7 ± 0.12	0.69 ± 0.11	−0.1 (−60–0.7)	0.455	0.608
eGFR (ml/min/1.73m^2)	119.2 ± 10.5	117.1 ± 11.7	−2.5 (−4–6)	0.367	118.7 ± 9.1	116.4 ± 9.7	−1.5 (−3–2.7)	0.318	0.297
UACR (mg/g creatinine)	97.5 (43–171.8)	18 (8.5–26)	−77 (−83–69)	<0.001	81.6 (42.7–157)	100 (65.1–123.3)	13 (−56–22)	0.068	<0.001
Cystatin C (ug/L)	852.5 ± 212.2	558.8 ± 140.4	−29 (−48–2)	<0.001	796.3 ± 384.3	961.8 ± 269.8	1.1 (−4–3)	0.217	<0.001
Vitamin B1 (ng/mL)	30.8 ± 7.4	57.8 ± 10.1	24 (19–32)	<0.001	35.1 ± 9.1	39.1 ± 8.7	5.4 (2.2–6.1)	0.147	<0.001
Vitamin B6 (ng/mL)	12.3 ± 4.6	31.7 ± 8.9	19.1 (9–27)	<0.001	15.6 ± 6.3	17.4 ± 7.8	6 (−7–10)	0.289	<0.001
Vitamin B12 (pg/mL)	314.9 ± 10.5	759.3 ± 52.9	130 (116–141)	<0.001	313.9 ± 10.7	312.5 ± 22.9	17 (−39–59)	0.747	<0.001
Homocysteine (μmol/L)	15.3 ± 3.6	6.2 ± 2.1	−14.1 (−19–6.1)	<0.001	14.8 ± 3.2	14.1 ± 3.4	3.6 (−1.8–5.6)	0.326	0.003

SDS: standard deviation score; BMI: body mass index; BP: blood pressure; FBG: fasting blood glucose; HDL cholesterol: high-density lipoprotein cholesterol; LDL cholesterol: low-density lipoprotein cholesterol; HbA1c: hemoglobinA1c; eGFR: estimated glomerular filtration rate; UACR: Urinary albumin creatinine ratio.

[a] P value was obtained using Analysis of covariance (ANCOVA) unless specified (*: Independent t-test was used; †: Chi-square test was applied).

[b] P value was obtained from paired-samples t tests for parametric variables or Wilcoxon rank-sum test for non-parametric variables.

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At study end, 5 patients dropped-out from the study because they did not attend follow-up measurements and were excluded from the final analysis; 2 participants were in vitamin B group and 3 were in placebo group, leaving 75 patients for the intention-to-treat analysis.

3.2. Effect of vitamin B complex supplementation on lipid profile

Vitamin B supplementation significantly lowered mean levels of triglycerides and total cholesterol while elevated HDL-cholesterol among vitamin B group compared with their baseline levels in the intervention group or with the levels at end of study among the placebo group (Table 1). This was also reflected on lowering systolic and diastolic blood pressure among vitamin B group compared with baseline levels or placebo group (Table 1).

3.3. Effect of B vitamins adjuvant therapy on kidney function and glycemic control

As shown in Table 1, at 12 weeks, vitamin B complex supplementation lowered glucose levels, improved glycemic control and significantly lowered the levels of homocysteine, UACR and cystatin C as compared with the placebo group at 12 weeks. Comparison of the percentage of change of UACR, cystatin C and homocysteine from baseline to 12 weeks between vitamin B and placebo groups showed significant differences ($p < 0.001$ for all comparisons). On the other hand, no significant difference was found in placebo group as regards HbA1c, UACR, cystatin C and homocysteine at 12 weeks compared with baseline levels. Supplementation with vitamin B complex was well tolerated and no side effects were reported throughout the study.

There were positive correlations between baseline cystatin C and each of systolic blood pressure ($r = 0.316$, $p = 0.047$), FBG ($r = 0.388$, $p = 0.013$), triglycerides ($r = 0.894$, $p < 0.001$), total cholesterol ($r = 0.816$, $p < 0.001$), HbA1c ($r = 0.413$, $p = 0.008$), UACR ($r = 0.611$, $p = <0.001$) and homocysteine ($r = 0.623$, $p = 0.005$) while cystatin C was negatively correlated to vitamin B12 ($r = -0.77$, $p = 0.001$; Fig. 2), vitamin B6 ($r = -0.312$, $p = 0.043$) and HDL-cholesterol ($r = -0.427$, $p = 0.025$) in DN patients with vitamin B adjuvant therapy. Multivariable linear regression analysis (Table 2) showed that baseline triglycerides, total cholesterol, HbA1c, UACR, vitamin B12 and homocysteine were the significant independent variables that affect cystatin C levels among patients with nephropathy receiving vitamin B supplementation.

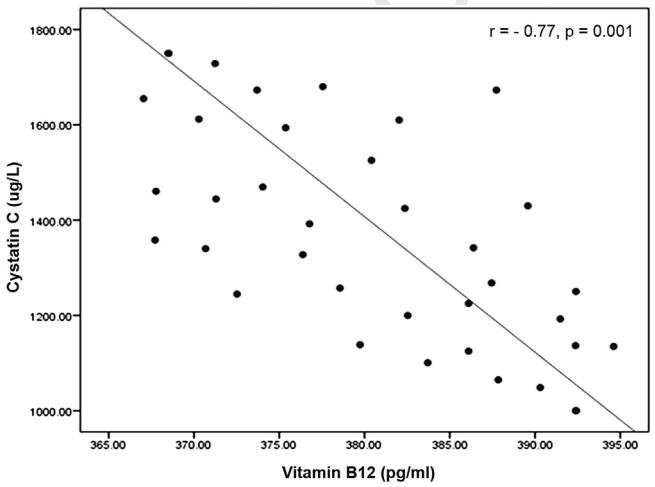

Fig. 2. Correlation between baseline cystatin C and vitamin B12 levels in diabetic nephropathy patients with vitamin B adjuvant therapy ($r = -0.77$, $p = 0.001$).

4. Discussion

Diabetes can alter the nutritional status and patients with diabetes are prone to deficiency of micronutrients such as magnesium, zinc, copper, manganese, and chromium. It was observed that serum ascorbic acid, B-vitamins, and possibly 1, 25-dihydroxycholcalciferol concentrations are low in patients with diabetes [35]. Evidence for deficiencies of vitamins B1, B6 and B12 in both T1DM and T2DM have been described in several publications [8–10,36,37]. An alternative etiology for deficiency of B vitamins in diabetic patients rather than nutritional factors may be the presence of oxidative stress which plays a major role in the pathogenesis and development of complications of both types of diabetes [38]. However, not much attention has been paid to the clinical evidence supporting this hypothesis, as well as to their possible therapeutic implications [39]. It is well known that antioxidant defense mechanisms involve both enzymatic and non-enzymatic strategies. Vitamins B1, B2, B6, and B12 are among the non-enzymatic antioxidants [40].

Given the enormous public health cost of diabetes, further studies are needed to explore the potential use of a relatively low-cost dietary supplement, such as vitamin B complex, as an adjuvant therapy in the prevention of complications [12]. The results of various previous studies have led to a growing awareness that the stage of DN, dose and duration of vitamin B complex supplementation are important determinants of outcome.

We preferred to give vitamin B supplementation as oral treatment to the studied type 1 diabetic children and adolescents instead of intramuscular (IM) injection because oral drugs are easily taken and more convenient in this age group especially that those patients are taking a minimum of 4 insulin injections/day. Previous studies used replacement therapy with either oral or parenteral vitamin B12 for patients with vitamin B12 deficiency and showed equal effect [41–43]. However, IM administration often involves a trip to a health facility or a home visit by a health professional to administer the injection and it can be painful [44]. Wang et al. [45] reported that oral and IM vitamin B12 have similar effects in terms of normalizing serum vitamin B12 levels and both are safe, but oral treatment costs less. Therefore, it should be the default treatment for vitamin B12 deficiency and that vitamin B12 oral tablets should be added to provincial drug plan formularies [43]. In adult patients with T2DM, IM or oral vitamin B12 in doses of 1000 μg daily for a week then once every week for 4 weeks were sufficient to correct vitamin B12 deficiency [41,42].

In our study, all included T1DM patients had microalbuminuria and none of them had macroalbuminuria. Supplementation with vitamin B complex (B1, B6 and B12) for 12 weeks lead to decline in UACR and cystatin C levels without adverse effects and vitamin B was an independent variable affecting cystatin C levels. Therefore, we could suggest that vitamin B complex was safe and effective adjuvant therapy for early stages of DN among pediatric T1DM patients. This reno-protective role of vitamin B complex in our patients is most likely related to improvement of hyperhomocysteinemia as shown by lower homocysteine levels in the intervention group after therapy.

The role of vitamin B in preventing microvascular complications of diabetes has already been a focus of research for some years especially in tissues where vascular complications develop [12]. Vitamin B1 has roles in improving endothelial cell function through the diversion of glucose flux from anaerobic to aerobic pathways, and therefore, may be important in delaying vascular complications of diabetes [19]. Incipient nephropathy was associated with more

Table 2
Multivariable linear regression analysis for factors affecting baseline cystatin C levels in type 1 diabetic patients with nephropathy who received vitamin B complex.

Independent variables	Unstandardized Coefficients		Standardized Coefficients	p value
	B	Standard Error	Beta	
Systolic BP SDS	46.15	40.05	0.184	0.256
FBG (mg/dL)	3.42	1.93	0.276	0.085
Triglycerides (mg/dL)	9.39	1.60	0.843	<0.001
Total cholesterol (mg/dL)	7.44	1.21	0.837	<0.001
HDL-cholesterol (mg/dL)	−29.4	12.86	−0.196	0.166
HbA1c (%)	325.14	122.10	0.397	0.011
UACR (mg/g creatinine)	1.74	0.48	0.505	0.001
Vitamin B6 (ng/mL)	−4.6	2.1	0.291	0.126
Vitamin B12 (pg/ml)	−10.26	1.06	−0.969	<0.001
Homocysteine (μmol/L)	20.4	8.7	0.421	0.003

BP: blood pressure; SDS: standard deviation score; FBG: fasting blood glucose; HDL cholesterol: high-density lipoprotein cholesterol; LDL cholesterol: HbA1c: hemoglobinA1c; UACR: Urinary albumin creatinine ratio.

pronounced alterations in vitamin B6 metabolism and stronger indications of endothelial dysfunction and inflammation [12].

Moreover, Koshy and collogues [36] estimated vitamin B12 in T1DM patients with a mean age 17.9 ± 4.96 years and showed that 45.5% of patients had low vitamin B12 using the manufacturer's cut-off of levels and 54% had low B12 using the published cut-off levels. The study did not demonstrate any significant correlation between vitamin B12 levels and age, duration of diabetes, diabetes control and no gender difference [36]. A high prevalence of vitamin B12 deficiency has been shown in patients with CKD [46]. Lack of vitamin B12 is thought to be a more important determinant for increased homocysteine which is related to the occurrence of micro-vascular complications [35]. Supplementation with vitamin B12 is known to reduce homocysteine levels [47].

In line with our results, Rabbani and collogues [48] conducted a study involving participants who were randomized to either placebo or oral high-dose thiamine (vitamin B1) supplementation of up to 300 mg/day for 3 months to assess whether such therapy could reverse microalbuminuria in T2DM with nephropathy. The investigators found a regression of UAE up to 41% from the baseline in the treatment group while there was no significant decrease in UAE in patients receiving placebo. Thus, it was concluded that thiamine supplements may provide improved therapy for early-stage DN [48]. Riaz et al. [32] reported that high-dose thiamine alone proved successful in decreasing UAE in the early stage of DN T2DM without improvement of glycemic control.

In contrast, a 12-week double-blind placebo-controlled trial in adult patients with T2DM and active DN found no effect of 300 mg three times daily of benfotiamine (a synthetic version of thiamine) on UAE or renal tubular damage markers [49]. The authors reported that thiamine derivatives may provide protective effects in earlier DN stages. This is in line with a previous animal study in which development of albuminuria after induction of diabetes was inhibited by thiamine and benfotiamine [2]. These results could explain the beneficial effect of vitamin B complex among our patients with T1DM who only had microalbuminuria, an early stage of nephropathy and we used vitamin B complex as an adjuvant therapy for short duration.

Of note, a multicenter, randomized, double-blind trial was done involving participants with DN with at least 300 mg/d of UAE (or ≥500 mg/d of proteinuria). Subjects received either a high-dose B-vitamin supplement (folic acid, vitamin B6, and vitamin B12) or placebo for 3 years. Participants in the therapy group had significantly lower plasma homocysteine levels but they experienced a rapid decline in renal function and had increased rates of vascular events such as myocardial infarcts and strokes [18]. The authors reported that this may be due to the use of high dose vitamin B therapy for long duration. At the beginning of the trial, serum levels of B vitamins were rather high among participants randomized to the B-vitamin group and by the end of the trial, they were very high. Mandatory folic acid fortification likely contributed to the observation of relatively high folate levels in all participants. The renal and vascular toxicity observed in their trial was explained by one or more of 3 potential mechanisms. First, folic acid may promote cell proliferation through its role in thymidine synthesis. Second, the use of folic acid and vitamin B12 might alter the methylation potential in vascular cells. Finally, B-vitamin therapy could potentially increase the methylation of L-arginine to the nitric oxide synthase inhibitor asymmetric dimethylarginine [50].

As regards vitamin B6, many studies showed that pyridoxamine inhibits the progression of renal disease, and decreases hyperlipidemia and apparent redox imbalances in diabetic rats [20,21,51]. Pyridoxamine and aminoguanidine had similar effects, supporting a mechanism of action involving the inhibition of advanced glycation end products and advanced lipoxidation end products (AGEs and ALEs) by trapping pathogenic reactive carbonyl compounds, the intermediates in the formation of AGEs [52]. Therefore, pyridoxamine delays the development of DN and reduces albuminuria in animal models of both type 1 and type 2 DN [20,53]. Moreover, pyridoxine supplementation restores normal glucose tolerance and reduces the thickening of the glomerular basement membrane [54] and has been shown to inhibit collagen cross-linking in a diabetic animal model [20].

Of interest, patients who received vitamin B complex showed a significant decrease in total cholesterol and triglycerides while HDL-cholesterol was significantly increased at end of therapy compared with their baseline levels in the intervention group or with the levels at end of study among the placebo group. This could be explained by the fact that vitamin B6 plays a vital role in the desaturation and elongation of fatty acids, methylation of phospholipids and mobilization of unsaturated fatty acids from triglycerides to phospholipids [55]. It has been reported that increased delta-desaturase activity stimulates prostaglandin E1 synthesis, which in turn inhibits cholesterol biosynthesis that modifies cholesterol levels [56].

Similarly, a 12 week, open-label, randomized, placebo-controlled trial investigated the effect of vitamin B6 supplementation (in a dose of 50 mg/day) and Lysine 1 g/day on the lipid profile and fasting plasma glucose level in Lebanese male patients with hypertriglyceridemia showed a reduction of plasma total cholesterol and HDL-cholesterol concentrations [57]. However, Polizzi et al. [58] reported that the combined administration of vitamins B1 and B6 to diabetic nephropathy patients causes a decrease in DNA glycation in leukocytes while administration of vitamin B6 alone did not have such effect. It is well known that advanced glycation plays an important role in the progression of diabetic complications.

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One limitation of this study is the small number of enrolled patients and therefore, these findings need to be confirmed in larger multicenter randomized trials to verify the role of vitamin B complex supplementation in treating early DN and for longer periods of time.

In conclusion, the positive correlation between cystatin C and homocysteine suggests that hyperhomocysteinemia may induce renal injury. Oral vitamin B complex supplementation once daily for 12 weeks as an adjuvant therapy to ACE-Is in children and adolescents with early stage of DN is safe, well tolerated and may have a nephro-protective action as it decreased the levels of homocysteine, albuminuria and cystatin C. Therefore, it could prevent the progression of DN. Moreover, it has a role in regulation of blood glucose homeostasis as reflected by lowering FBG and HbA1c as well as improving dyslipidemia. In our study, the benefit of vitamin B complex supplementation was observed in type 1 diabetic patients with micro-albuminuria and relatively good glycemic control. However, it remains to be determined if severely uncontrolled diabetic patients could benefit from such treatment. Further studies with elongation of the duration of vitamin B complex supplementation for better result profiling and long-term effects on vascular pathology and kidney disease progression are warranted and represent an interesting area for future research.

Conflict of interest

Nothing to declare.

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Appendix A. Supplementary data

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