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Original Article

Serum micronutrient levels in children and adolescents with type 1 diabetes mellitus

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Abstract

Several factors such as stringent dietary practices and occult malabsorption disorders may compromise the nutrition of children with T1DM, but the data regarding the dietary status of Indian T1DM children is limited. Hence, this study was conducted to assess serum and blood levels of vitamins, minerals, and essential trace elements in children and adolescents with T1DM. Twenty T1DM (serum IgA tissue transglutaminase antibody-negative) and 20 age-matched apparently healthy children were included in the study. Blood samples were collected in the fasting state to estimate serum vitamins (except vitamin C), macrominerals, zinc, copper, and iron, and whole blood essential trace elements (cobalt, chromium, manganese, molybdenum, nickel, selenium, tin, vanadium). The analysis revealed comparable age, sex, height standard deviation score (SDS), and weight SDS between the two groups. There were no significant differences in the levels of any of the vitamin level except for higher serum vitamin B5 (32.5±10.8 *vs.* 26.27±6.25 ng/ml, p = 0.018), blood manganese levels (13.95±4.23 *vs.* 11.78±1.65 ng/ml, p=0.039) and lower blood tin levels (0.99±0.36 *vs.* 1.33±0.35 ng/ml, p=0.005) in T1DM individuals than controls. All subjects had hypovitaminosis D (25-hydroxy vitamin D: 10–30 ng/ml). To conclude, most of the serum vitamins, minerals, and essential trace element levels of T1DM children and adolescents are comparable to controls. Most Indian children and adolescents, including those with T1DM, require supplementation with vitamin D.

Keywords: type 1 diabetes mellitus, children, vitamins, minerals, micronutrients.

Introduction

Type 1 diabetes mellitus (T1DM) is the second most common chronic disease in Indian children, with an annual incidence of 3.7–4 cases [1]. Medical nutrition therapy (MNT) plays an important role in glycaemic management and growth optimization in T1DM children. The nutritional status of T1DM children may be compromised due to associated comorbidities such as celiac disease, autoimmune gastritis, autonomic neuropathy, eating disorders, mild exocrine pancreatic insufficiency, or overzealous dietary restrictions for glycaemic control. These factors may also lead to micronutrient deficiency in children with T1DM, which is not a well-known phenomenon. An Indian study has reported reduced dietary intake of a few micronutrients among T1DM children and adolescents [2], whereas few studies from other countries have reported lower vitamin levels among T1DM patients [3–7]. However, no Indian studies have documented the status of micronutrients in patients with T1DM based on serum micronutrient levels. Hence, we have measured serum levels of vitamins and minerals in T1DM children and adolescents and compared them with age and sex and matched controls.

Material and methods

This cross-sectional study was conducted in the Department of Endocrinology, Narayana Medical College and Hospital, Nellore, Andhra Pradesh, India. The institutional ethics committee of Narayana Medical College (NMC/Adm/Ethics/approval/003/05/2018) approved the study. Written informed consent of the parents or guardians of all participants and consent of all children over the age of seven were obtained. The study was in accordance with the World Association Code of Ethics (Declaration of Helsinki).

Children and adolescents with T1DM aged 3 to 18 years attending the Outpatient Clinic of the Department of Endocrinology were screened for the study. Apparently healthy children and adolescents in the same area were recruited as controls. Participants with vitamin supplementation in the previous three months (n=1), celiac disease (n=0), untreated or sub-optimally treated hypothyroidism (n=1), chronic kidney disease (n=1) and chronic liver disease (n=0) were excluded from the study.

A nutritionist took a detailed diet history (3 days diet recall), and all participants were examined for clinical manifestations of nutrient deficiencies. Anthropometric measurements (height, weight, and body mass index (BMI) were obtained, and the respective z-scores were calculated using the revised IAP growth charts data [8]. Blood samples were collected after overnight fasting for at least eight hours.

Plasma glucose, serum creatinine, calcium, phosphorus, magnesium, sodium, chloride, and liver function tests were measured using an automated biochemistry analyzer (Humastar 600, Human Diagnostics), and HbA1c was measured by high-performance liquid chromatography (Biorad D10). Serum IgA tissue transglutaminase and IgA were measured by enzyme-linked immunosorbent assay, whereas thyroid function tests were analyzed by chemiluminescence immunoassay. Serum/blood levels of all vitamins and minerals were measured at Thyrocare®, Mumbai. Serum vitamin B12 analysis was performed by chemiluminescent immunoassay on the Advia Centaur XPT immunoassay system, Siemens Healthineers.

Serum levels of all other vitamins were measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS). The water used for the mobile phase preparations was deionized to type 1 purity. Sample treatment reagents used were methanol (Optima™ LC/MS Grade, Fisher Scientific), ethanol (EMSURE® ACS, ISO, Reag. Ph Eur), formic acid 99% (Carlo Erba ACS - Reag. Ph.Eur. - Reag. USP.), chloroform (Carlo Erba GCMS grade ISO - ACS - Reag. Ph.Eur. - Reag. USP), and trichloroacetic acid >99% (ACS reagent). Mobile phases were 0.1% formic acid in water and 0.1% formic acid in methanol. Commercially available calibrators and controls of Recipe ClinChek® and Zivak were used to analyze vitamins A, D2, D3, E, K, and B6, while serum spiked calibrators and controls were used for the other B complex vitamins. To analyze vitamins A, D2, D3, E, and K, 100 µl of serum sample was added to 400 µl of 7:1 ethanol-chloroform mixture followed by vortex mixing and centrifugation; 50 µl of supernatant was injected into the LC-MS system. To analyze vitamins B1, B2, B3, B5, B7, and B9, 200 µl of serum sample was added to 600 µl of methanol followed by vortex mixing and centrifugation; 5 µl of supernatant was injected into the LCMS instrument. For vitamin B6 analysis, 200 µl of serum sample was added to 200 µl of 5% trichloroacetic acid in water (w/v). The mixture was vortex mixed and centrifuged; 5 µl of supernatant was injected into the LC-MS/MS instrument for analysis. Analysis was performed on a Shimadzu Nexera X2 UHPLC autosampler coupled with an 8045 Triple quadrupole mass spectrometer (Shimadzu, Kyoto, Japan). Vitamins A, D2, D3, E, and K were separated on a Phenomenex Kinetex C8 50mm*4.6mm chromatography column and B complex vitamins were separated on a Phenomenex C18 75mm*4.6mm column. Analytes were detected on multiple reaction monitoring modes (MRMs) and quantified based on their specific mass-to-charge (m/z) transitions.

Serum zinc and copper were measured using a Beckman Coulter AU5800 photometry analyzer. Serum zinc and copper levels were measured using the Dialab kits based on the colorimetric 5-Br-PAPS method and colorimetric 3.5-Dibromo-PAESA method, respectively. Other trace element levels in whole blood were measured using inductively coupled plasma mass spectrometry (ICP-MS). A whole blood sample (3–5 ml) was collected into K3-EDTA tubes (trace element free). All solvents and reagents used were of the highest commercially available purity grade.

Deionized water was used to prepare all standard solutions and samples. Suprapur grade 65% HNO_3 (Honeywell) was used for sample dissolution. Multi-element, MS grade stock solutions of Co, Cr, Mo, Mn, Ni, Se, Sn, and V were used for standard preparations. A mono-element MS grade Yttrium, Rhodium, and Iridium solution from Elemental Scientific were used as an internal standard. The purity of the Argon for plasma generation and nebulizing was >99.99%.

120 µL of the blood sample was mixed with 2800 µL diluent (5% HNO_3+2% Gold Solution). Samples were spiked with the internal standard Yttrium, Rhodium, and Iridium before digestion to monitor the recovery of the elements. Thermo ScientificTM ICP-MS iCAPQ series (Thermo Scientific, Germany) equipped with a hexapole collision cell (CC) coupled with an Elemental Scientific SC-4 DX Autosampler (Omaha, NE, USA) was used for element quantification. The sample introduction system consisted of a Peltier cooled (3°C) baffled cyclonic spray chamber, PFA nebulizer, and quartz torch with a removable 2.5 mm ID quartz injector. The instrument was operated on kinetic energy discrimination (KED) mode using ultrapure Helium in the collision or reaction cell (CRC).

Statistical analysis

Data were analyzed using SPSS for windows 22.0 (Armond, NY, IL). Categorical variables were expressed as frequency or percentages, whereas continuous variables were expressed as mean±SD. Continuous variables between the two groups were compared using Student's t-test, whereas categorical variables were compared using Fisher's exact test. For all analyses p-value of <0.05 was considered statistically significant.

Results

The study included 20 children and adolescents with T1DM and 20 healthy children and adolescents. All except one child with T1DM had received at least one dietician consultation in the last six months. The duration of diabetes among T1DM participants was 3.48±1.1 years. There were 13 boys and seven girls in each group. Age, gender distribution, height z-score, and weight z-score were not different between the two groups (Table 1).

Serum levels of vitamin A, E, K, and D were not significantly different between children and adolescents with T1DM and healthy controls (Table 1). Two controls and one T1DM child had slightly low vitamin K levels (<0.1 ng/ml). All participants had 25OH-vitamin D levels between 10–30 ng/ml; 11 controls and nine cases had 25OH-vitamin D levels between 20 and 30 ng/ml

Table 1: Comparison of demographic characteristics and serum vitamin levels between children and adolescents with type 1 diabetes mellitus and controls.

	Controls (n=20)	Type 1 diabetics (n=20)	P-value
Age (years)	11.65±4.15	12.35±4.73	0.62
Sex (Male: female)	13:7	13:7	1.00
Height z-score	-0.53±0.83	-0.5±1.07	0.92
Weight z-score	-0.34±1.22	-0.34±1.06	0.99
Vitamin A (ng/ml)	358.22±73.32	387.42±81.87	0.253
Vitamin E (ng/ml)	9379.02±1834.86	9981.10±1143.86	0.221
Vitamin K (ng/ml)	0.30±0.24	0.29±0.17	0.889
Vitamin D2 (ng/ml)	0.56±0.39	0.58±0.60	0.902
Vitamin D3 (ng/ml)	18.10±4.69	19.39±4.75	0.393
Vitamin D (ng/ml)	18.67±4.78	19.98±4.86	0.396
Thiamine (ng/ml)	0.80±0.30	0.76±0.56	0.761
Riboflavin (ng/ml)	33.42±20.36	32.18±16.98	0.835
Nicotinic acid (ng/ml)	0.615±0.29	0.47±0.22	0.08
Pantothenic acid (ng/ml)	26.27±6.25	32.50±10.08	0.018
Pyridoxal 5 phosphate (ng/ml)	13.48±4.45	15.25±6.00	0.295
Biotin (ng/ml)	0.92±1.51	0.71±0.49	0.552
Folic acid (ng/ml)	0.31±0.09	0.34±0.08	0.261
Vitamin B12 (pg/ml)	357.33±104.16	348.92±218.73	0.888

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(vitamin D insufficiency), whereas the remaining were vitamin D deficient (25OH-vitamin D: <20 ng/ml). Serum levels of vitamin B1, B2, B3, B6, B7, B9, and B12 were not significantly different between children and adolescents with T1DM and healthy controls (Table 1). Serum pantothenic acid (B5) levels were significantly higher in children and adolescents with T1DM than in controls. Two controls had slightly low serum thiamine levels (<0.5 ng/ml), whereas all other water-soluble vitamin levels were normal in all participants.

Serum calcium, phosphorus, magnesium, and chloride levels were not significantly different between children and adolescents with T1DM and healthy controls (Table 2). Serum sodium levels were significantly lower in children and adolescents with T1DM, although both groups had levels within the normal range without any hyponatremia or hypernatremia.

Blood tin levels were significantly lower, whereas manganese levels were significantly higher in T1DM children and adolescents than in controls (Table 2). Serum iron levels tended to be lower in T1DM cases than in controls. However, TIBC and transferrin saturation were comparable between the groups.

Discussion

We reported serum vitamin and mineral levels in Indian children and adolescents with T1DM. Although limited by a smaller sample size, this is the first study that has reported the serum levels of most of the vitamins and minerals in T1DM children and adolescents compared to age- and sex-matched controls.

Serum levels of fat-soluble vitamins were comparable between children and adolescents with T1DM and healthy controls. Similarly, few studies have reported comparable serum retinol [9] and α-tocopherol levels in T1DM individuals and controls [10]. In contrast, several previous studies have reported lower serum retinol [3, 11] but variable (higher or lower) α-tocopherol levels

Table 2: Comparison of serum mineral levels between children and adolescents with type 1 diabetes mellitus and controls.

Minerals	Controls (n=20)	Type 1 diabetics (n=20)	P-value
Serum calcium (mg/dl)	9.94±0.38	9.68±0.64	0.135
Serum albumin (g/dl)	4.27±0.20	4.12±0.38	0.130
Serum phosphorus (mg/dl)	5.20±0.81	5.30±0.79	0.719
Serum magnesium (mg/dl)	2.03±0.13	1.97±0.15	0.183
Serum sodium (mEq/L)	141±1.19	138±3.50	0.0008
Serum chloride (mEq/L)	105±2.20	104±3.31	0.26
Serum iron (µg/dL)	81.71±32.89	62.79±26.56	0.066
Serum total iron binding capacity (TIBC) (µg/dL)	427.80±65.26	398.18±55.86	0.151
Transferrin saturation (%)	9.57±8.56	16.29±7.53	0.228
Serum copper (µg/dL)	1.27±21.69	1.40±19.63	0.067
Serum zinc (µg/dL)	85.67±22.2	88.82±19.1	0.63
Serum chromium (ng/ml)	0.72±0.42	0.89±0.61	0.321
Serum cobalt (ng/ml)	0.53±0.14	0.51±0.22	0.734
Serum tin (ng/ml)	1.33±0.35	0.99±0.36	0.005
Serum molybdenum (ng/ml)	1.29±0.73	1.24±0.38	0.780
Serum vanadium (ng/ml)	0.61±0.28	0.59±0.30	0.840
Serum selenium (ng/ml)	115.93±22.30	121.10±25.58	0.500
Serum nickel (ng/ml)	1.43±0.34	1.31±0.36	0.257
Serum manganese (ng/ml)	11.78±1.65	13.95±4.23	0.039

in T1DM individuals than in controls [9, 11, 12]. Serum levels of vitamin K were low to normal in most participants in both groups, with unclear causes.

Several previous studies have reported lower vitamin D status in T1DM individuals than in controls [3, 13, 14]. However, vitamin D status between T1DM individuals and controls was comparable in our study, also observed in the Pediatric Diabetes Consortium cohort [15]. Notably, hypovitaminosis D was universal in our study participants. The observation suggests a need to ensure measures to improve vitamin D status in children and adolescents from the region, especially in those with T1DM, as it helps not only to optimize bone health but may also help in the optimization of glycaemic control.

Serum pantothenic acid level was significantly higher in children and adolescents with T1DM than in healthy controls; but the exact reason for this difference is not known as there was no difference in the dietary intake of the vitamin between the groups (4.16±0.93 *vs.* 4.7±1.16, p=0.11). Serum levels of vitamin B1, B2, B3, B6, B7, B9, and B12 were not significantly different between children and adolescents with T1DM and healthy controls. In contrast, few previous studies have reported lower thiamine levels in T1DM individuals, especially in those with diabetic ketoacidosis [6, 7], whereas the reports regarding serum folate and vitamin B12 are highly variable with lower, comparable as well as higher levels in T1DM patients than controls [16–19].

There was no significant difference in the serum levels of microminerals between children and adolescents with T1DM and healthy controls except for serum sodium. The lower sodium level in individuals with T1DM was most probably due to the coexistent hyperglycemia, as the glucose-corrected sodium levels did not differ between the groups [20]. Serum iron levels tended to be lower in T1DM patients than controls but not TIBC. This may suggest a tendency for latent iron deficiency in T1DM patients. However, serum ferritin levels were not measured, which could have better-reflected iron stores in the body. Few studies have demonstrated frequent absolute and functional iron deficiency among T1DM children and adolescents [21]. The common reasons for iron deficiency among T1DM children, such as celiac disease, hypothyroidism, and renal dysfunction, were ruled out in our patients, whereas there was no significant difference in iron intake between T1DM patients and controls.

Blood tin levels were significantly lower in our patients with T1DM than in controls. However, no previous study has evaluated tin levels in patients with T1DM and the exact cause for tin deficiency and its implications in T1DM patients are unclear. Blood manganese (Mn) levels were significantly higher in patients with T1DM than in controls. In contrast, the only previous study comparing Mn status between T1DM children and healthy controls revealed a tendency for lower blood Mn levels in T1DM children with significantly lower levels among those with microvascular complications [22].

Serum zinc (Zn) levels were comparable between cases and controls, as reported in an Iranian study [23]. Serum copper (Cu) levels were also not different between cases and controls. In contrast, some studies have reported elevated serum copper levels in T1DM patients [24, 25]. A few studies have also documented an increased Cu and Zn ratio in T1DM patients and its positive correlation with HbA1c [25].

The small sample size limited the study. Plasma vitamin C level was not measured in our study since it was not offered in the vitamin profile used in our study. For the assessment of deficiencies of a few vitamins, measurement of serum vitamin levels are not reliable indicators. For example, measurement of vitamin-dependent enzymes and demonstration of improvement in their activity after replacement (thiamine and riboflavin) or measurement of urinary metabolites (nicotinamide) are more reliable markers of vitamin deficiency than serum vitamin levels. However, such measurements were not part of our study [26]. Hence, there is a need for larger studies that estimate micronutrient status among Indian T1DM children and adolescents using more reliable nutritional biomarkers.

Conclusions

Serum/blood levels of most vitamins and minerals are comparable between T1DM children and adolescents and controls. Most South-Indian children and adolescents, including those with T1DM, suffer from hypovitaminosis D; hence, they require supplementation with vitamin D.

Conflicts of interest

The authors declare no conflict of interest.

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