Evaluation of Serum Vitamin B12 level in Egyptian Children and Adolescents with Type 1 Diabetes

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ABSTRACT

B12 deficiency causes permanent debilitating peripheral neuropathy, balance and gait disorders, tremors, frequent falls, and visual disturbances. It also shares many of the outcomes as diabetes, by attacking the autonomic nervous system (i.e. gastroparesis, impotence, urinary incontinence). However, there is insufficient data regarding association between vitamin B12 deficiency and T1D in the Egyptian population. This study aimed to find out an association between vitamin B12 deficiency and T1D in Egyptian population attending a primary care unit, to assess the need for regular screening and supplementation of vitamin B12.

Subjects and Methods: The study was performed as a case control study, and was conducted in El Arab school health insurance unit in port-Said city. The study included 40 diabetic patients as study group and 40 healthy control group. Both groups were subjected to complete history, physical examination, and peripheral blood samples were withdrawn for Serum vitamin B12 level, diabetic profile (Fasting, random blood sugar and HbA1c) and anti-parietal cell antibody, (APCA) APCA was estimated by ELISA, for exclusion of PA (for group A only). The data were collected, revised and summarized, the results were tabulated and analysed.

Conclusions: there is no association between vitamin B12 deficiency, and type 1 diabetes in children and adolescents apart from pernicious anemia and there is no relation between serum vitamin B 12 level and duration of diabetes, diabetic control, age or gender of type 1 diabetes children and adolescents. So, there is no need for routine screening or supplementation of vitamin B12 for children and adolescents with Type 1 diabetes.

Keywords: Vitamin B12; Vitamin B12 insufficiency; Type 1 Diabetes Children adolescents

Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. Type 1 diabetes mellitus (T1DM) (Insulin Dependent Diabetes Mellitus: IDDM) results from autoimmune destruction of insulin producing beta cells and is characterized by the presence of insulitis and beta cell auto antibodies. It is associated with other autoimmune endocrine disorders and auto antibodies leading to the development of autoimmune polyglandular syndrome [2]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels [3]. Vitamin B12 or cobalamin is a water-soluble vitamins that have a fundamental role in DNA synthesis, erythropoiesis and nerve function. Therefore, clinical features of vitamin B12 deficiency hence, are predominantly those of hematological and neurocognitive dysfunction [4]. If left untreated, B12 deficiency causes permanent debilitating peripheral neuropathy, balance and gait disorders, tremors, frequent falls, and visual disturbances. Chronic B12 deficiency also causes demyelination of the nerves and spinal cord which may lead to partial or complete paralysis. B12 deficiency also shares many of the outcomes as diabetes, by attacking the autonomic nervous system (i.e. gastroparesis, impotence, urinary incontinence). It can also cause optic neuropathy and atrophy, as well as retinal disease [5,6]. Worsening of diabetic neuropathy is also noted among patients with co-existing vitamin B12 deficiency [7]. Previous literature reports that B12 deficiency strikes 16% of the population, or 48 million Americans. Others report the prevalence to be 25%. The Framingham Offspring Study found that nearly 40% of people age 26 to 83 had B12 levels in the "low normal" range—a level at which many begin experiencing neurological symptoms [8]. Vitamin B12 deficiency due to pernicious anemia (PA) occurs frequently among patients

with T1DM. In one cross-sectional study done in South India among 90 patients with T1DM, low vitamin B12 levels were noted among 45.5% of the study subjects as defined by the manufactures' cut off point of <180 pg/ml and among 54% using the published cut off point of <200 pg/ml [9]. Presence of parietal cell antibodies (PCA) and antibodies to intrinsic factor have been demonstrated in type 1 diabetes (T1D) patients. These factors could contribute to the occurrence of B12 deficiency in these patients. In addition, the dietary habits which vary from one population to another could also contribute to the deficiency [10]. Biochemical and clinical vitamin B12 deficiency has been demonstrated to be highly prevalent among patients with type 1 and type 2 diabetes mellitus (T2DM) [11]. There is a high prevalence of vitamin B complex deficiency among patients with T1D that is connected to diabetic nephropathy," Elbarbary said. "It would be pragmatic to screen for it in the presence of any clinical manifestations to avoid diabetic microvascular complications." – by Regina Schaffer [12]. However, there is insufficient data regarding association between vitamin B12 deficiency and T1D in the egyptian population. Hence this study was undertaken to evaluate serum B12 levels in type 1 diabetics in egyptian population attending a primary care unit, to assess the need for regular screening and supplementation of vitamin B12.

Patients and Methods:
This study was conducted along period from October 2018 to June 2019, at El Arab school health insurance unit in port-said city. The study was performed as case control study, to study the association between vitamin B12 deficiency and T1D to assess the need for regular screening and supplementation of vitamin B12 in type1 diabetics.

Inclusion criteria: Patients with T1D aged less than 18 years and exclusion criteria: Patients who are strict vegetarians. Patients with history of malabsorption syndromes, previous gastrectomy. Patients on drugs known to interfere with vitamin B12 absorption, such as phenytoin, dihydrofolate reductase inhibitors, etc. Patients with positive anti- parietal cell antibodies. Group B (control): Subjects for the control group were taken from healthy non-diabetic age and gender matched subjects. The studied subjects were divided into 2 groups: Group A (Type 1 diabetics) n= 40 and Group B (Control) n= 40.

Both groups were subjected to: Full history: Adequate history taking through interview with one or both parents and the health care team including: Personal Data: Name, age, gender. Diabetes history: including time of diagnosis, regimen of treatment and complications if present Past medical history: Any chronic illness, operations.

Drug history: as certain medications may contribute to vitamin B12 deficiency namely proton pump inhibitors and metformin [13]. Nutritional history: to exclude vegetarians. Symptoms of vitamin B12 deficiency: Weakness, tiredness, or lightheadedness, palpitations, shortness of breath, pallor, loss of appetite, nerve problems like numbness or tingling, muscle weakness, and mental problems like depression, memory loss, or behavioral changes. Neurological examination for neuropathy. Signs of vitamin B12 deficiency: eg. Glossitis, progressive weakness, ataxia, and paresthesias that may progress to spasticity and paraplegia Signs of uncontrolled hyperglycemia: eg. Candidal infection.

Laboratory nvestigations:
(Were performed at Suez Canal University Hospital Lab)Sampling: 5 ml sample was collected by venipuncture, whole blood was divided into two tubes; an EDTA containing tube for HbA1c and plain tube for the other laboratory tests, blood was allowed to clot and serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at – 20 C. Serum Vitamin B12 level was estimated by fully automated electrochemiluminescence immunoassay cobas e411 (Roche Diagnostics, Germany). Diabetic profile (Fasting, random blood sugar and HbA1c), was performed by Cobas c 501.

APCA was estimated by ELISA, for exclusion of PA. (for group A only)

Operational Definitions:
T1D was diagnosed based on history, clinical evaluation and laboratory findings. The American Diabetes Association criteria were used for the diagnosis. It includes the following: (American Diabetes Association, 2021). FPG level ≥126 mg/dL (7.0 mmol/L), or

A 2-hour PPG ≥200 mg/dL (11.1 mmol/L) during a 75-g OGTT, or

A random plasma glucose ≥200 mg/dL (11.1 mmol/L) in a patient with classic symptoms of hyperglycemia or hyperglycemic crisis. HbA1C measurement: ≥ 6.5 percent. Vitamin B12 levels were evaluated based on a published meta-analysis report which considers a value less than 148 pm ol/L (200 pg/mL) as deficient [14].

Statistical Analysis:
Data were collected then tabulated and analyzed using computer facilities. Statistical analysis was performed according to standard methods using Statistical Package of Social Science (SPSS) version 17.

Count, percent, Mean and standard deviation were estimated for each continuous variable. Chi-square test and independent t-test were used to assess the statistical difference between variables, each test according to the type of variable. Statistical significance was considered at P-value <0.05. Study results were described in tables and graphs.

Statistical Analysis Procedures:
Analysis of variance (F-test): All data was subjected to statistical analyses of variance (F-test) "one way ANOVA". It is a procedure used for testing the differences among the means of two or more treatments. It was noted that if means of subgroups are greatly different, the variance of the combined groups is much larger than the variance of the separate groups. The analysis of variance format for the analysis of differences in means is based on this fact. Duncan's multiple range tests is one of the multiple comparison's procedures. It uses the "t" distribution corresponding to the number of degrees of freedom for error mean square. The significance of the measured data was considered as follows (N.S.) when P>0.05 Not significant while P<0.05 Significant Where P is the probability (reflect of null hypothesis) (Armitage, 1971). Cross tabs (X2): The Crosstabs procedure forms two-way and multi way tables and provides a variety of tests and measures of association for two-way tables. The structure of the table and whether categories are ordered determine what test or measure to use. Cross tabs statistics and measures of association are computed for two-way tables only. If you specify a row a column and a layer factor (control variable), the Crosstabs procedure forms one panel of associated statistics and measures for each value of the layer factor (or a combination of values for two or more control variables [15].

Statistical analysis: Data were collected then tabulated and analyzed using computer facilities. Statistical analysis was performed according to standard methods using Statistical Package of Social Science (SPSS) version 17. Count, percent, Mean and standard deviation were estimated for each continuous variable. Chi-square test and independent t-test were used to assess the statistical difference between variables, each test according to the type of variable. Statistical significance was considered at P-value <0.05. Study results were described in tables and graphs.

Results

This Case control study was conducted in El Arab school health insurance unit in port-said city, to estimate serum vitamin B12 levels in type 1 diabetics. The study included 40 diabetic patients as study group and 40 others without diabetes as control group. We compared the general characteristics between both groups as regarding gender and age. There were no statistically significant differences between both groups (P-value >0.05), that means both groups were matched. The Mean ± SD of the Duration of DM was 4 ± 2.

We compared the Laboratory investigations between both groups. The study group showed higher HbA1c, FBS and RBS, and these differences were statistically significant (P-value <0.05). However, B12 and Hb showed no statistically significant differences between both groups (P-value >0.05), as shown in table (1).

Table 1: Laboratory investigations of the studied participants (n=80).

Laboratory investigations		Study group (n=40)	Control group (n=40)	P-value
HbA1c	Mean ± SD	8.5 ± 2	5 ± 0.3	0.001*
	Median (min-max)	8.3(5.9-14)	5(4.4-5.6)	
FBS	Mean ± SD	142 ± 56	82 ± 8	0.001*
	Median (min-max)	131(78-350)	81.5(70-99)	
RBS	Mean ± SD	221± 84	128 ± 17	0.001*
	Median (min-max)	193(135-470)	129.5(94-156)	
B12	Mean ± SD	687 ± 186	655 ± 138	0.38
	Median (min-max)	720(222-1056)	664.5(389-895)	
Hb	Mean ± SD	10.9 ± 1	10.6 ± 1	0.279

*P-value is statistically significant

We compared the clinical data between both groups as regarding Vitamin B12 deficiency and Neurological ex. There were no statistically significant differences between both groups (P-value >0.05), However, positive Correlations between B12 and other variables except for Hb were found among the studied participants as shown in table (2).

Table 2: Correlations between B12 and other variables among the studied participants (n=80).

Correlations			Age	HbA1c	FBS	RBS	Hb
Case	B12	Pearson Correlation	-0.178	-0.194	-0.152	-0.005	-0.083
		P-value	0.271	0.231	0.348	0.974	0.61
		N	40	40	40	40	40
Control	B12	Pearson Correlation	-0.185	0.079	0.053	0.186	-0.15
		P-value	0.253	0.627	0.745	0.25	0.356
		N	40	40	40	40	40

Diabetes is a group of metabolic diseases characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both [1]. Type 1 diabetes mellitus (T1DM) results from autoimmune destruction of insulin producing beta cells and is characterized by the presence of insulitis and beta cell auto antibodies [2]. Vitamin B12 or cobalamin is a water-soluble vitamin that have a fundamental role in DNA synthesis, erythropoiesis and nerve function [3]. This case control study was conducted in El Arab school health insurance unit in port-said city, to estimate serum vitamin B12 levels in type 1 diabetics, aiming to assess the need for regular screening and supplementation of vitamin B12 in type1 diabetics, as underestimation or excess supplementation without need may be harmful. The study included 40 diabetic patients as study group and 40 others without diabetes as control group.

Vitamin B12 deficiency due to pernicious anemia (PA) occurs frequently among patients with T1DM [15]. In addition, the dietary habits which vary from one population to another could also contribute to the deficiency [2]. B12 deficiency causes permanent debilitating peripheral neuropathy, balance and gait disorders, tremors, frequent falls, and visual disturbances. Chronic B12 deficiency also causes demyelination of the nerves and spinal cord which may lead to partial or complete paralysis. B12 deficiency also shares many of the outcomes as diabetes, by attacking the autonomic nervous system (i.e. gastroparesis, impotence, urinary incontinence) [5,6]. As these effects are serious, and contribute to morbidity in a diabetic patient it is important to be detected and treated if found. Worsening of diabetic neuropathy is also noted among patients with co-existing vitamin B12 deficiency [7]. The Framingham Offspring Study found that nearly 40% of people age 26 to 83 had B12 levels in the "low normal" range—a level at which many begin experiencing neurological symptoms [8].

Unfortunately, the symptoms of diabetic neuropathy overlap with the paresthesias, impaired vibration sense, and impaired proprioception associated with B12 deficiency. As a result, B12 deficiency–induced nerve damage may be confused with or contribute to diabetic peripheral neuropathy [16]. Therefore, identifying the correct etiology of neuropathy is crucial because

simple vitamin B12 replacement may reverse neurologic symptoms inappropriately attributed to hyperglycaemia [9]. On the other hand excess of vitamin B6 (pyridoxine) -which is present in most vitamin B complex preparations- is harmful; sensorimotor neuropathy secondary to pyridoxine toxicity can be debilitating [17]. Vitamin B6 toxicity produces a sensory ataxia, areflexia, and impaired cutaneous sensation. Patients often complain of burning or paresthesias. Electrodiagnostic testing usually shows a sensory neuronopathy, but with severe toxicity motor nerves can be affected as well. Symptoms of toxicity can be seen with doses as low as 100 mg per day [18]. There is insufficient data regarding association between vitamin B12 deficiency and T1DM in the egyptian population. Also it is noticed that vitamin B12 supplementation (mostly as vitamin B complex preparations) was given routinely for all type 1 diabetic children, in most health insurance care units without screening. Hence this study was undertaken to evaluate serum B12 levels in type 1 diabetics in egyptian population attending a health insurance care unit, to assess the need for regular screening and supplementation of vitamin B12 in type1 diabetics. The study group, patients were tested first for APCA, and only those who tested negative were included. APCA was chosen for exclusion of PA as they are present in most cases, about 85-90% [19,20], and therefore it is an adventitious tool for screening of PA [21].

General characteristics in both groups as regarding gender and age were matched. The average age of patients was 9yrs, so old age is excluded as a risk factor for vitamin B12 deficiency, as in general, vitamin B12 level declines with age and prevalence of vitamin B12 deficiency increases with age [22].

https://www.hkmj.org/abstracts/v21n2/155.htm - r1 The mean FPG was 142 mg/dL, while the RBS was 221 mg/dL and the mean HbA1c was 10.13%.

All patients of the study group showed normal vitamin B12 levels. The mean serum vitamin B12 level was 687pg/ml. There was no statistically significant difference between both groups regarding serum vitamin B12 levels. Only one patient had a borderline vitamin

B12 level (222pg/ml). Other similar study that was conducted in a tertiary care hospital in India reported that the prevalence of low serum vitamin B12 was found to be 45.5%. Out of them, 28.5% had values in the deficient range while 17% were in the indeterminate range [9].

This contrary results may be attributed to difference in sociodemographic factors of both population groups, although age of studied population was comparable, but their study sample consisted of patients from various regions of Karnataka registered with the Bangalore Diabetes Registry, who followed different dietary habits (mostly strict vegeterians), and a detailed dietary history was not available [9], so B12 deficiency may be due to nutritional deficiencies while in our study adequate intake of vitamin B12 from dietary sources was ensured. Also the presence of PCA was not assessed in the other study, so it's role can't be excluded, while in our study cases with positive PCA were excluded from the study.

Our results were matching with that of a case control study done in Italy which was done on T1D patients with mean age of 16.7 yrs, and mean diabetes duration of 8.4 yrs, where serum vitamin B12 levels were normal in all patients, with mean levels of 698 pg/ml in females and 832 pg/ml in males [23].

Our results were similar also to that of another case control study done in Turkey which included 32 children with type 1 diabetes and 23 age and sex matched healthy children, aiming to to determine plasma total Hcy levels, to determine correlates of plasma total Hcy levels with nutritional factor such as serum folic acid and vitamin B12 levels. Serum vitamin B12 levels were within normal limits in all study subjects with mean 551 pg/ dl, and levels were not different between both groups [24].

This was also comparable to a study done in diabetology department in a university hospital in Poland aimed to assess the prevalence of ATP4A autoantibodies in pediatric T1D patients and to explore the relationship between ATP4A positivity and vitamin B12 concentrations, which showed that mean vitamin B12 levels in ATP4A negative patients was 474 pg/ml, which is normal and also comparable to our study result [25].

In another study done in diabetes clinic in a tertiary care hospital in Edinburg, on T1D patients with autoimmune thyroid disease, with median duration of diabetes was 22yrs, the median serum vitamin B12 concentration was 442 ng/l which was also comparable to our study. Four of sixty three studied patients (6.3%) had low serum B12 concentrations, of which three of them had positive IF antibody, and only one patient had low serum B12 with negative IF antibody (1.5%) [26]. This is near to our study in that vitamin B12 deficiency is not prevalent in T1D patients apart from pernicious anemia. The slight difference may be attributed to different age population as median age of patients was 54yrs which was much higher than our study population, and it can't be excluded as a contributing risk factor. Up to 38% of older adults may exhibit mild vitamin B12 deficiency and depleted vitamin B12 stores [27], as the capacity to absorb vitamin B12 from a food-based diet decreases in older adults and over time can result in the food-cobalamin malabsorption syndrome, characterized by mild vitamin B12 deficiency, decreased whole body stores and metabolic disturbances [28]. The mean duration of diabetes in the studied group in our study was 4 yrs, ranging from 0.4 to 11yrs duration. There was no statistically significant correlation between B12 level and duration of diabetes in the studied group. This is similar to what was found in another study in India where no correlation between B12 and the duration of diabetes was found [9]. Moreover, in our study, there were no statistically significant correlations between B12 and other variables; age, HbA1c, FBS, RBS and Hb level among the studied participants. This was similar the other study in India as there was also no correlation between B12 and the diabetic control or age [9]. In another case control study done in Italy, on 41 patients (21 males and 20 females) with T1DM, all were Caucasians, vitamin B12 levels were significantly higher only in male patients than in male controls [23]. This difference may be related to different ethnic group of studied population or nutritional habits. All patients with low serum B12 concentrations were asymptomatic, and have no clinical evidence of neurologic abnormality [26].

Limitations of the study

Other markers for vitamin B12 deficiency such as MMA and holo TC were not evaluated, due to high cost. Elevated methylmalonic acid and homocysteine levels improve the diagnosis of tissue B12 deficiency, especially in cases with borderline B12 levels [29].

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