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Original Article

Evaluation of the effects of diabetes mellitus and metformin usage on serum vitamin B12 levels in cobalamin deficient subjects

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Abstract

Introduction. Although there are studies evaluating vitamin B12 levels in different patient groups, there is none encountered in Turkish adult patients with or without DM reported in the English literature. The aim of the study was to evaluate the possible additional effects of diabetes and metformin usage on serum levels of vitamin B12 in cobalamin deficient Turkish adult patients.

Methods. Medical records of subjects ≥18 years of age, having a vitamin B12 level <180 pg/mL were screened, consecutive 98 subjects were included in the study.

Results. Among a total of 75 female and 23 male subjects with a mean age of 51.3±15.9 years and vitamin B12 level of 139.3±29.2 pg/mL, 34 had the diagnosis of type 2 diabetes mellitus and 64 had no diabetes diagnosis. Mean ages were 59.0±10.8 years for diabetics and 47.2±16.8 years for nondiabetics. Vitamin B12 levels were found to be insignificantly low in people with the diagnosis of diabetes compared to without diabetes (131.2±30.6 and 143.5±27.7 pg/mL, respectively, p=0.05). Vitamin B12 levels had no correlation with diabetes duration, presence of complications, metformin usage duration.

Conclusions. In conclusion, our results demonstrated that people with diabetes had lower levels of vitamin B12 compared to nondiabetics, but this fact could not solely be explained by the duration of disease, accompanying complications, metformin treatment duration. All patients with or without the diagnosis of diabetes should be encouraged for sufficient vitamin B12 intake and all possible factors that lead to deficiency should be eliminated.

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Introduction

Vitamin B12 or cobalamin is mainly found in animal origin food products. It has important roles in many different biochemical reactions.[1] The minimum daily requirement of vitamin B12 is 6 mcg and a Western type diet contains 5 to 7 mcg of cobalamin.[2] Total body store of vitamin B12 is 2 to 5 milligrams. Main storage is in the liver being approximately one-half. A serum vitamin B12 level of <200 pg/mL is consistent with cobalamin deficiency with a specificity of 95-100%.[3] Insufficient cobalamin intake due to strict vegan diets, insufficient cobalamin absorption due to malabsorption syndromes, hereditary transcobalamin II deficiencies and some drugs like metformin, proton pump inhibitors (PPI) and histamine 2 receptor antagonists may lead to vitamin B12 deficiency. The frequency of vitamin B12 deficiency varies between 3 to 40 % in different populations. Inadequate intake is the main problem in most populations with low socioeconomic level.[4,5] Metformin is the first-choice therapy for people with type 2 diabetes mellitus (DM). It improves insulin resistance and decreases cardiovascular risk with its efficacy and favorable safety profile. Besides it has some side effects, one of them being vitamin B12 malabsorption.[6-10] The mechanisms leading to vitamin B12 deficiency due to metformin in patients with type 2 DM are not clear. Alterations in small bowel motility, bacterial flora and intrinsic factor levels and competitive inhibition of the calcium dependent absorption of vitamin B12- intrinsic factor complex are supposed to be the main problems in metformin usage.[10-12] Although there are many studies in the literature evaluating vitamin B12 levels in different patient populations including those with DM and the effect of metformin usage on vitamin B12 levels in the groups with DM.[1,5,7,9-12], we could not encounter a study conducted and reported in the English literature in Turkish adult patients with or without DM. Our present study was carried out in Turkish adult patients with detected vitamin B12 deficiency, with or without the diagnosis of DM, to evaluate the possible additional effects of DM and metformin usage on serum levels of vitamin B12.

Methods

This was a retrospective cross-sectional study carried out in Endocrinology and Metabolism outpatient clinic of a university hospital during 1-year period. The study was performed with the approval of the local ethics committee and in accordance with the Declaration of Helsinki.

Medical records of subjects 18 years of age or older, having lower than normal vitamin B12 level, with or without the diagnosis of DM and for DM patients with or without metformin usage were screened. Ninety-eight consecutive subjects were included in the study. Vitamin B12 level less than 180 pg/mL which was the lowest limit of the normal range according to our laboratory was taken as the limit value for cobalamin deficiency. Data of the patients were obtained through a retrospective screening of the patient files. Age, gender, height, weight, body mass index (BMI), autoimmune disease status, other accompanying diseases and medications were recorded in all subjects. In the DM group presence, duration, type and complications of DM and dosage and duration of metformin usage were evaluated since all of these factors were shown to affect vitamin B12 levels in different studies in the literature.[9-12] As the laboratory data, fasting blood glucose, hemoglobin A1c (HbA1c), vitamin B12 levels were recorded from the files. Fasting blood glucose level was measured with photometric method using Abbott 16000 device, HbA1c was measured with high performance chromatography using Adams A1c HA 8160 device and vitamin B12 level was measured with chemiluminescence method using Abbott Architect device. Normal ranges for fasting blood glucose were between 70-100 mg/dL, HbA1c were 4.0-6.1% and vitamin B12 were 180-1162 pg/mL.

Statistical analysis

All statistical analysis was done with statistical package program SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Shapiro-Wilk test of normality was used to verify distribution of the variables. The categorical variables were expressed as actual numbers with percentages and the continuous variables as mean ± standard deviation. Unpaired Student's two sampled t test or Mann-Whitney U-test were used to compare two groups. Nonparametric data were analyzed using Kruskal–Wallis test among three groups. In

case of significance, the difference was confirmed by Mann–Whitney test. The significance level was determined as $p<0.05$.

Results

A total of 75 (76.5%) female subjects and 23 (23.4%) male subjects were included in the study. Mean age of all subjects was 51.3±15.9 years. Mean age for women was 50.4±15.5 years and men 54.3±17.2 years. Mean BMI was determined to be 27.8±6.2 kg/m2 for whole population, 29.1±6.5 kg/m^2 for women and 24.6±3.8 kg/m^2 for men. Mean value for vitamin B12 level was determined to be 139.3±29.2 pg/mL for whole population, 140.9±28.9 pg/mL for women and 133.8±30.1 pg/mL for men.

DM diagnosis was available in 34 of the patients included in the study all being type 2. Sixty-four subjects were without diabetes. Mean age of subjects without DM was 47.2±16.8 years and with DM was 59.0±10.8 years. Mean vitamin B12 levels of subjects without DM (n=64) and with DM (n=34) were compared. Mean vitamin B12 level in subjects without DM was 143.5±27.7 pg/mL (median=148.5 pg/mL, min=83 pg/mL, max=179 pg/mL) and with DM 131.2±30.6 pg/mL (median=134.0 pg/mL, min=81 pg/mL, max=177 pg/mL). Vitamin B12 levels were found to be low in subjects with DM compared to others but this difference was statistically insignificant (p=0.05) (Table 1, Figure 1).

Table 1. Age, body mass index and vitamin B12 values of all group and subjects with and without diabetes

Variables	All group (n=98)	Without diabetes mellitus (n=64)	With diabetes mellitus (n=34)
Age (year)	51.3±15.9	47.2±16.8	59.0±10.8
Body mass index (kg/m^2)	27.8±6.2	26.9±6.4	28.9±5.9
Vitamin B12 (pg/mL)	139.3±29.2	143.5±27.7	131.2±30.6*

*: p=0.05, when compared with the group without diabetes mellitus

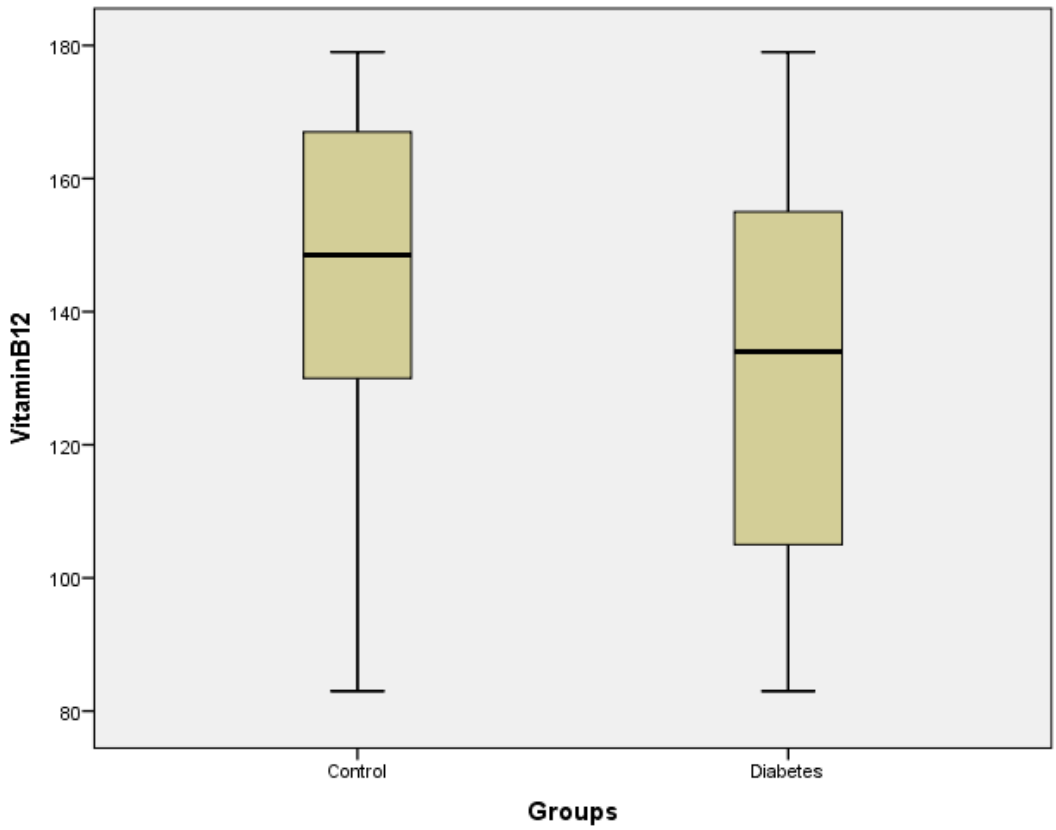

Figure 1. Vitamin B12 levels for subjects with and without diabetes mellitus (p=0.05)

Table 2. Age, body mass index and vitamin B12 values of all subjects with diabetes with and without diabetic complications*

Variables	All subjects with diabetes mellitus (n=34)	Without diabetic complications (n=18)	With diabetic complications (n=16)
Age (year)	59.0±10.8	55.7±8.9	62.8±11.7
Body mass index (kg/m^2)	28.9±5.9	28.2±6.9	29.5±5.2
Vitamin B12 (pg/mL)	131.2±30.6	126.1±33.1	137.0±27.5

*:p>0.05

There were accompanying illnesses other than DM in whole group. Thyroid abnormalities without autoimmunity including multinodular goiter were present in 22 patients (22.4%), dyslipidemia in 16 (16.3 %), Hashimoto thyroiditis in 16 (16.3%), hypertension in 10 (10.2%), hirsutism in 5 (5.1%), hyperprolactinemia in 5 (5.1%), gastric problems in 4 (4.0%), chronic kidney disease in 3 (3.0 %), panhypopituitarism in 3 (3.0%), osteoporosis in 2 (2.0%), heart failure in 2 (2.0%), parathyroid adenoma in 2 (2.0%), stomach cancer in 1 (1.0%) and lung cancer in 1 (1.0%) patient. Among comorbidities, as being autoimmune, Hashimoto thyroiditis can be together with insufficient absorption of vitamin B12. When subjects with and without Hashimoto thyroiditis were compared, no statistically significant difference was detected between 2 groups concerning vitamin B12 levels (142.6±32.5 vs 138.6±28.7 pg/mL, respectively, p=0.489). In the group with DM only 3 had accompanying Hashimoto thyroiditis.

The patients with DM diagnosis were categorized into 3 groups according to diabetes duration as being less than 5 years, 5 to 10 years and more than 10 years. The number of patients with diabetes duration less than 5 years was 12 (35.2%), between 5 to 10 years was 16 (47.0%), and 10 years or longer was 6 (17.6%). When vitamin B12 level was evaluated according to diabetes duration, it was 125.5±31.6 pg/mL in patients with less than 5 years duration, 131.5±32.3 pg/mL in patients between 5-10 years duration and 142.0±25.2 pg/mL in patients with 10 years or longer duration. Vitamin B12 levels were comparable among three groups. There was also no significant correlation between known disease duration and vitamin B12 levels in 34 patients with diabetes mellitus (r=0.160, p=0.367).

When diabetes related complications were evaluated 18 (52.9%) subjects out of 34 had no microvascular complications. One (2.9%) patient had diabetic nephropathy, 2 (5.8%) had diabetic retinopathy, 3 (8.8%) had diabetic neuropathy, 4 (11.7%) had diabetic gastropathy, 6 (17.6%) had multiple diabetic microvascular complications. There was no significant difference between vitamin B12 levels in patients with or without diabetic complications (p=0.578). Age, body mass index and vitamin B12 values of all subjects with diabetes, with and without diabetic complications are given in Table 2.

Mean fasting blood glucose levels of diabetics involved in the study was computed to be 128.1±8.3 mg/dL and mean HbA1c was 6.3±0.2%. They were well regulated subjects concerning glycemic parameters. Thirty DM patients were using metformin treatment, and all were receiving metformin in a dosage of 2 grams per day. Eleven (32.3%) patients were using metformin for less than 5 years, 15 (44.1%) patients between 5 to 10 years and 4 (11.7%) patients for 10 years or longer. The other antidiabetic medications used in combination with metformin were sulfonylureas, glinides, glitazones, alpha-glycosidase inhibitors, dipeptidyl peptidase 4 inhibitors and insulins. When vitamin B12 level was evaluated according to metformin duration, it was 133.3±30.1 pg/mL in patients with metformin usage less than 5 years, 137.2±32.3 pg/mL in patients with metformin usage between 5-10 years and 130.2±19.7 pg/mL in patients with metformin usage 10 years or longer. When duration of metformin usage was considered, vitamin B12 levels did not differ (p=0.280).

There were drugs used by the subjects other than antidiabetics. Among all subjects 26 (26.5%) were using levothyroxine, 9 (9.1%) lipid lowering agents, 5 (5.1%) PPIs, 3 (3.0%) antihypertensives and 37 (37.7%) multiple drugs. Among the drugs only PPIs were shown to affect absorption of vitamin B12. Vitamin B12 levels of two groups

using PPI or not were comparable (137.8±17.0 vs 139.3±29.8 pg/mL, respectively, p= 0.657).

Discussion

In this study, we evaluated the possible additional effects of DM and metformin usage on serum levels of vitamin B12 in cobalamin deficient patients with DM and compared them with those of without DM. The prevalence of vitamin B12 deficiency differs among different studies.[1,10,13,14] The prevalence is reported to be less in people living actively within the society (12%) and higher in those who are older and reside at the care homes (30-40%).[13] Similarly, the prevalence of cobalamin deficiency in the Framingham elderly population was reported to be 20%.[14] The prevalence of vitamin B12 deficiency in patients with long term metformin usage was reported to be 5.8-30% in different studies.[1,10] The mean age of our patients was 51 years who were living actively in the population.

The causes of vitamin B12 deficiency can be divided into three groups as nutritional deficiencies, gastrointestinal malabsorption syndromes and other causes.[15] DM is a metabolic disorder affecting many people worldwide, and its complications are important including autonomic neuropathies.[16] Many factors; including suboptimal intake, contribute vitamin B12 deficiency in people with or without DM. Low intake may be the result of low socioeconomical levels and preferring vegetarian diets. People with DM may have additional defects in vitamin B12 absorption as a result of motility problems due to gastroparesis and absorption problems due to autoimmune comorbidities other than metformin usage. Fortunately, even in situations of serious malabsorption, people store as much vitamin B12 as to be adequate for the following two and five years.[15] Although Hashimoto thyroiditis is an autoimmune disorder, we could not demonstrate its effect on vitamin B12 levels. Our subjects with and without Hashimoto thyroiditis had similar levels of vitamin B12. This result might be due to the small number of subjects in our study.

Being diabetic and having diabetic complications like autonomic neuropathy and diabetic gastroparesis may be risk factors for developing vitamin B12 deficiency. In our study, overall group was having low vitamin B12 levels with a mean value of 139.3±29.2 pg/mL. Besides diabetic people had lower vitamin B12 levels compared to nondiabetics (131.2±30.6 vs 143.5±27.7 pg/mL) which was statistically insignificant. On the other hand, we could not demonstrate any correlation between vitamin B12 levels and diabetes duration and presence of diabetic complications.

According to American Diabetes Association (ADA) and The Society of Endocrinology and Metabolism of Turkey (SEMT), first line oral antidiabetic agent to be initiated in newly diagnosed type 2 diabetic patients is metformin.[8,10,16] It has the opportunity of lowering insulin resistance which is the core problem in many type 2 diabetics. Most of our diabetic patients were using metformin in accordance with the ADA and SEMT guidelines. All of them were using maximum effective dose of metformin 2 grams/day alone or in combination and had good glycemic control with the mean fasting blood glucose of 128 mg/dL and mean HbA1c level of 6.3%. Metformin has advantages of experience in long term usage, lower cost and excellent reliability reports. Nevertheless, it has been reported that long term use of metformin may lead to side effects particularly on the vitamin B12 metabolism.[9,10,16]

It is known that decrease in vitamin B12 levels can start as early as 4th month of metformin usage. Clinical features of deficiency usually appear by 5 years due to body stores which can be affected by age and metformin dosage used.[11] In a study conducted, during a 4.3 year follow up of type 2 diabetic patients who received metformin and insulin treatment, vitamin B12 level had decreased approximately 19%.[17] A study conducted in type 2 diabetics indicated that metformin usage >4 years and average daily dose >1000 mgs are at increased risk for vitamin B12 deficiency.[10] Unlike these results, we could not demonstrate any correlation between vitamin B12 levels and duration of metformin usage in the diabetic group in our study. We also could not demonstrate any correlation between vitamin B12 levels and PPI usage.

There are some limitations of our study. It was a small sample sized retrospective study indicating that people with type 2 DM had

lower level of vitamin B12 compared to ones without DM but diabetes duration and presence of complications, duration of metformin usage were not contributors. The inconsistency with the results of the studies from the literature might be due to small sample size which may lead to type II statistical error and comparisons might not reach statistical significance. Our results should be confirmed in studies with larger patient numbers. On the other hand, the inconsistency with the results of the studies from the literature might also highlight the importance of factors other than metformin usage like low vitamin B12 intake in people with DM as well as without DM. Since type 2 DM usually coexists with dyslipidemia dietary restrictions in cholesterol might limit the intake of animal products rich in vitamin B12.

Conclusions

Our results demonstrated that although statistically insignificant, people with type 2 DM had lower levels of vitamin B12 compared to nondiabetics but this fact could not solely be explained by the duration of disease, accompanying complications, metformin treatment duration. As should be done in whole population, people with DM should also be courage for sufficient vitamin B12 intake and other possible factors that can aggravate the deficiency should be eliminated. Keeping the possible unfavorable effect of metformin on vitamin B12 in mind, we should be aware that all people with or without DM can be a candidate for vitamin B12 insufficiency and care must be given to whole population to prevent the problem.

Conflict of interest

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

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