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Original Article

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Vitamin B12 Status Among Patients With Type 2 Diabetes Mellitus

Mamoun Mukhtar[a], Yehia S. Mohamed[b, c, h], Murtada Elbagir[a], Yohannes Tesfa[a], Sami Kenz[a], Vijay Kumar[a], Sijomol Skaria[a], Mohamed A. Baraka[d, e], Ghassan Darwiche[f], Akrem Elmalti[g]

Abstract

Background: Vitamin (vit.) B12 is a vital nutrient for ideal hemopoietic, neurocognitive, and cardiovascular integrity. The frequency of vit. B12 abnormalities in patients with type 2 diabetes mellitus (T2DM), especially with the chronic use of metformin, has been demonstrated and verified. In this study, the levels of vit. B12 and its metabolites in metformin-using T2DM participants were measured and compared to healthy participants to conclude any causal relationship.

Methods: The study was conducted on selected T2DM patients who are using a high dose of metformin for the long term and compared to healthy volunteers regarding the following parameters: body mass index (BMI), waist circumference, complete blood cell (CBC), serum homocysteine, methylmalonic acid, transcobalamin, and holo-transcobalamin. SPSS was used for data analysis, implementing the unpaired Student's *t*-test and the Chi-square test for significance.

Results: Levels of holo-transcobalamin and homocysteine were insignificantly higher in T2DM patients. However, transcobalamin was insignificantly higher in the test group, while methylmalonic acid was surprisingly below the normal levels among both groups. No significant difference in BMI was reported between both groups, but T2DM patients had significantly higher waist circumferences compared to their healthy counterparts.

Conclusions: The study showed high homocysteine levels in T2DM patients, consistent with the literature of similar studies of vit. B12 deficiency. However, it also showed unexplained reduced levels of methylmalonic acid and unexpectedly high levels of transcobalamin and homo-transcobalamin in the T2DM patients compared to the control group. These results indicate additional investigations are needed using a larger sample size of both groups to reach a meaningful conclusion.

Keywords: Vitamin B12 deficiency; Metformin; Type 2 diabetes mellitus; Vitamin supplementation

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[a]Rashid Center for Diabetes and Research, Sheikh Khalifa Medical City - Al Jurf, Ajman, UAE
[b]Department of Pathological Sciences, College of Medicine, Ajman University, Ajman, UAE
[c]Department of Microbiology and Immunology, Faculty of Pharmacy (Boys), Al-Azhar University, Nasr City, Cairo, Egypt
[d]Pharmacy Department, Fatima College of Health Sciences, Abu Dhabi, UAE
[e]Clinical Pharmacy Department, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo, Egypt
[f]Department of Clinical Sciences, Faculty of Medicine, Lund University, Malmo, Lund, Sweden
[g]Dr. Suliman Habib Hospital, Dubai, UAE
[h]Corresponding Author: Yehia S. Mohamed, Department of Pathological Sciences, College of Medicine, Ajman University, Ajman, UAE.
Email: yehiasmohamed@hotmail.com

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Introduction

The micronutrient vitamin (vit.) B12, sometimes referred to as cobalamin, is essential for the synthesis of DNA components, hemopoiesis, and neurological metabolic processes. Hematological and neurocognitive impairment are the main clinical signs of vit. B12 insufficiency [1].

Animal protein is the most important source of vit. B12. In the stomach, the intrinsic factor (IF; a parietal cell-released glycoprotein) forms a complex with vit. B12 enabling its intestinal absorption. Following IF degradation, vit. B12 is released freely within the enterocytes. This free vit. B12 binds to an intracellular protein carrier, transcobalamin II (TCII), and the complex is subsequently moved to the bloodstream. Bone marrow, liver, and other tissues actively absorb vit. B12-TCII complex, also known as holo-transcobalamin (HTC). Up to 90% of the absorbed vit. B12 is stored predominantly in the liver [2]. Disruption in any of the absorption or transportation processes results in clinical or sub-clinical deficiency in vit. B12.

Vit. B12 catalyzes homocysteine methylation to methionine, which is then converted to S-adenosyl-methionine which is a methyl group donor to myelin, neurotransmitters, and membrane phospholipids. The frequency of vit. B12 insufficiency in adults under the age of 65 is from 6% to 12%, and in adults with macrocytic anemias, approximately 17%. Elderly, patients regularly using metformin, proton pump inhibitors (PPIs), or H2 blockers, pregnant women, and vegans are more vulnerable to vit. B12 deficiency due to a lack of absorption or the physiological high demand. Therefore, a better index

of suspicion should be applied to those populations when suggestive symptoms and signs exist. It is reported that vit. B12 deficiency interrupts the methylation process and causes the buildup of homocysteine in tissues, which has potential toxic effects on vascular endothelium and neurons as well. Moreover, vit. B12 enables transformation of methyl-tetrahydrofolate to tetrahydrofolate, the active form of folate. It also catalyzes the transformation of methyl malonyl coenzyme A (CoA) to succinyl-CoA in an additional crucial biologic pathway. This process is diminishing in cases of vit. B12 deficiency, leading to increased serum methylmalonic acid (MMA).

All of the aforementioned factors explain the peripheral neuronal, neurocognitive, and hematological consequences of vit. B12 insufficiency [3, 4]. In addition, the associated hyperhomocysteinemia is connected with an increased risk of heart and vascular consequences [5-7]. Vit. B12 deficiency is also associated with macrocytic anemia (mean corpuscular volume (MCV) > 100 fL), ovalocytes, hyper-segmented neutrophils, and pancytopenia [8].

Numerous observational studies [9, 10] and case reports [11-13] have reported an elevation in the prevalence of vit. B12 insufficiency in patients with type 2 diabetes mellitus (T2DM), especially with the chronic use of metformin [14-17]. It is reported that vit. B12 insufficiency in metformin-taking T2DM patients ranges from 5.8% to 33% [17, 18], and this substantial variation in the reported prevalence is likely caused by the variable descriptions of vit. B12 insufficiency in different settings.

Globally, metformin is considered the first-line oral anti-hyperglycemia in conjunction with lifestyle changes [19-21]. In spite of the glucose-lowering effect of metformin, it has long been documented to reduce vit. B12 levels too. In an early study conducted by Dr. DeFronzo's group, metformin was reported to reduce serum vit. B12 levels by 22% versus placebo and 29% versus glyburide. Several consequent cross-sectional studies [9, 10], case reports [11, 13], and randomized controlled trials [14, 15] have demonstrated the downsides of metformin too.

The risk of evolving metformin-associated vit. B12 insufficiency is significantly influenced by the age, dose, and length of metformin use [15, 16]. In a previous study involving 155 Chinese T2DM patients taking metformin, and 310 healthy volunteers, the results disclosed that for each 1 g/day metformin dosage was related to 2.9% (95% confidence interval (CI)) increased risk of vit. B12 insufficiency. Metformin typically causes a reduction in the absorption of vit. B12 and its serum concentration within less than 4 months [22], and clinical symptoms appear within 5 - 10 years due to the huge hepatocyte reservoir [23].

The speculated strategies of metformin-related vit. B12 include modifications in intestinal peristalsis, which results in microbial overgrowth and subsequently vit. B12 loss; competitive blocking or inhibition of absorption; changes in IF concentrations; or blocking the receptor of cubilin endocytosis [23]. Metformin was also reported to block calcium-dependent vit. B12-IF internalization in the terminal part of the ileum. However, with calcium administration, this blocking effect is overturned [24].

The measurement of blood homocysteine or MMA levels is a specific and sensitive screening method, particularly in T2DM patients with vit. B12 levels between 200 and 400 pg/mL and associated with subtle hematological features. The normal range for homocysteine and MMA concentrations in serum is 5 - 15 µmol/L and 0.28 µmol/L, respectively [25-27]. As the first line for management of T2DM, it is important to consider metformin-associated deficiency of vit. B12. This cross-sectional study was conducted at the Rashid Center for Diabetes and Research (RCDR), a tertiary health care center in Ajman, which serves the population of the Northern Emirates of the UAE, to investigate the incidence of vit. B12 insufficiency in the local population.

Materials and Methods

This observational cross-sectional study was conducted at RCDR over 1 year from May 1, 2018 to April 20, 2019. More than 8,000 patients with diabetes mellitus of both types are registered and followed up in this health care facility.

Ethical considerations

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the Emirati Ministry of Health and Prevention's Research Ethics Committee with reference number: MOHP/REC-9/2018.

Study population

Adult T2DM patients (aged 18 - 65) visiting the center for regular follow-up during the study period and meeting the inclusion criteria were invited to participate as the study group. Adult healthy volunteers (aged 18 - 65) from Sheikh Khalifa Medical City Ajman (SKMCA) staff members and co-patients with no history of diabetes or vit. B12 deficiency were invited to participate as the control group.

Inclusion and exclusion criteria

Males or females with T2DM, taking a daily metformin dose of ≥ 2,000 mg for ≥ 2 years, and with estimated glomerular filtration rate (eGFR) ≥ 30 mL/min/1.72 m^2, were selected for the study. Patients on vit. B12 supplement or with known pathological vit. B12 deficiency due to partial or total gastrectomy, atrophic gastritis, inflammatory bowel disease, celiac disease, or any other malabsorption condition were also excluded. In addition, patients on long-term PPIs, H2 receptor blockers, calcium supplements, or having severe liver disease with prothrombin time > 1.1, untreated hypothyroidism, or current malignancy were also excluded from the study.

Biochemical analysis

The complete blood cell (CBC) analysis was performed on the same day of collection with Sysmex KX-21N (Bioprom,

Table 1. Demographics, Clinical Characteristics and Lab Test Results for T2DM Patients and Controls (n = 353)

Characteristic	Patients (n = 263)	Controls (n = 90)	P-value
Age (years)	53.0 ± 8.6	38.3 ± 10.3	< 0.001*
Male (n, %)	108 (41%)	21 (22%)	0.001*
Female (n, %)	155 (59%)	69 (78%)	0.001*
BMI (kg/m^2)	32.2 ± 6.8	32.9 ± 6.7	0.360
Waist circumference (cm)	103.0 ± 15.3	99.3 ± 11.1	0.015*
Diabetes history duration (years)	12.0 ± 6.8	NA	
SBP (mm Hg)	132.0 ± 14.0	123.3 ± 13.2	< 0.001*
DBP (mm Hg)	72.0 ± 9.1	70.4 ± 10.4	0.132
eGFR (mL/min/1.73 m$^{2)}$	90.0 ± 22.0	108.0 ± 14.0	< 0.001*
Serum creatinine (µmol/L)	61.5 ± 18.2	59.2 ± 11.7	0.172
TSH (mU/L)	2.1 ± 1.2	2.1 ± 1.9	0.653
Transcobalamin (pmol/L)	271.4 ± 117.6	261.7 ± 101.0	0.450
Homocysteine (µmol/L)	11.5 ± 3.9	9.8 ± 2.4	< 0.001*
Holo-transcobalamin (pmol/L)	81.3 ± 33.2	68.1 ± 31.4	0.002*
Methylmalonic acid (ng/mL)	2.6 ± 2.0	4.9 ± 2.2	< 0.001*[a]
Hb (g/L)	130.6 ± 18.3	127.2 ± 17.1	0.114
MCV (fL)	78.4 ± 7.2	79.0 ± 7.0	0.516
HCT (vol%)	40.0 ± 4.9	38.7 ± 3.9	0.003*
WBC (× 10^9/L)	7.5 ± 1.8	6.4 ± 1.7	< 0.001*
Neutrophils (× 10^9/L)	4.0 ± 1.5	3.4 ± 1.2	< 0.001*
Platelets (× 10^9/L)	265.0 ± 81.5	278.4 ± 72.1	0.158

Comparisons between groups using unpaired Student's *t*-test. Data are shown as mean ± standard deviation. The distribution of gender among groups is expressed as numbers (%) of males versus females. *P < 0.05. [a]In 138 subjects (117 patients and 21 controls) data on methylmalonic acid were missing. BMI: body mass index; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; Hb: hemoglobin; HCT: hematocrit; MCV: mean corpuscular volume; SBP: systolic blood pressure; T2DM: type 2 diabetes mellitus; TSH: thyroid-stimulating hormone; WBC: white blood cell.

Greece). Serum samples for vit. B12 analyses (transcobalamin and HTCII) were stored at -20 °C and tests were performed weekly with Roche Cobas 6000, e-601 (Roche, USA). Serum samples for homocysteine, MMA and IF antibodies were tested as batches using Abbexa ELISA Kits in Human Elisys Quattro and plates were read using automated ELISA reader (Diagnostica Wiesbaden, Germany). The other renal and thyroid function testes were reported by the hospital chemistry lab.

Statistical analysis

Patient sample size was determined to be 283 using Andrew Fisher's formula at a 95% confidence level (z-score 1.96), a standard deviation (SD) of 0.5 and a CI of ±5%, and normal healthy participants were 101. Sample size was calculated from the following formula: $(\text{z-score})^2 \times SD \times (1 - SD)/CI^2$.

Values are given as mean ±SD. Test results of patient and control groups were interpreted using unpaired Student's *t*-test or Chi-square test based on the data type. P-value < 0.05 was considered significant. Linear regression analysis for age and gender was carried out to further study the differences between the two groups regarding serum levels of transcobalamin, homocysteine, HTC and MMA. All analysis was performed using SPSS 26.0 (IBM Corporation, NY, USA).

Results

This study aimed to explore the effects of metformin treatment on vit. B12 levels among T2DM patients who have been using high-dose (≥ 2,000 mg daily) metformin for ≥ 2 years. Out of the 283 patients and 101 control participants, 20 patients and 11 controls were excluded from the study due to an undisclosed intake of supplements (reported HTC was ≥ 650 pmol/L). The average age in years of the patient group is 53.0 ± 8.6 (n = 263), with female to male participants at 59% (n = 155) and 41% (n = 108), respectively. The average age in years of the control group is 38.3 ± 10.3 (n = 90), with 78% (n = 70) female to 22% (n = 20) male participants (Table 1).

No significant difference was reported in BMI in the patient compared to the control participants (P = 0.360). However, waist circumference was significantly higher in the patient (103.0 ± 15.3) compared to the control (99.3 ± 11.1) groups

Table 2. Analysis of Transcobalamin, Homocysteine, Holo-Transcobalamin and Methylmalonic Acid in Diabetes Patients and Controls (n = 353)

Parameter	Patients (n = 263)	Controls (n = 90)	P-value
Transcobalamin (pmol/L)	271.4 ± 117.6	261.7 ± 101.0	0.475
Homocysteine (µmol/L)	11.5 ± 3.9	9.8 ± 2.4	0.368
Holo-transcobalamin (pmol/L)	81.3 ± 33.2	68.1 ± 31.4	0.527
Methylmalonic acid (ng/mL)	2.6 ± 2.0	4.9 ± 2.2	< 0.001*[a]

Comparisons between the two groups using linear regression analysis controlled for gender and age. Data are shown as mean ± standard deviation. *P < 0.05. [a]In 138 subjects (117 patients and 21 controls) data on methylmalonic acid were missing.

(P = 0.0151) (Table 1). Systolic blood pressure readings were also significantly higher (in mm Hg) among the patient (132.0 ± 14.0) compared to healthy control (123.0 ± 13.2) (P < 0.001) (Table 1). The mean duration of T2DM history among the patient group in years was 12.0 ± 6.8.

eGFR was significantly lower among the patients compared to the control group (P < 0.001); however, the average values in both groups were within the normal range. Serum creatinine and thyroid-stimulating hormone (TSH) results showed no significant difference between both groups.

The analysis of the biochemical and hematological laboratory parameters is summarized in Table 1. Forty subjects (31 patients (11.8%) and nine controls (10%)) were reported to have low values of transcobalamin (below 145 pmol/L), 25 subjects (15 patients (5.7%) and 10 controls (11.1%)) had low values of HTC (below 38 pmol/L), and 24 subjects (22 patients (8.4%) and two controls (2.2%)) showed elevated levels of homocysteine (above 15 µmol/L). By running a Pearson Chi-square test, the results were significantly different between the patient and the control groups for homocysteine (P = 0.024), but not for transcobalamin and HTC. None of the participants had a high abnormal value for MMA (the normal range is 20 ng/mL). Even though the mean level of serum transcobalamin was close in the patient and control groups (271.4 ± 117.6 vs. 261.7 ± 101.0), respectively (P = 0.450), levels of homocysteine (11.5 ± 3.9 vs. 9.8 ± 2.4) (P < 0.001) and HTC (81.3 ± 33.2 vs. 68.1 ± 31.4) (P = 0.002) were significantly greater in the patient group than the control one (Table 2) [28]. On the other side, MMA concentrations were significantly lower in the patient group (2.6 ± 2.0 vs. 4.9 ± 2.2) (P < 0.001). Due to the discrepancy between the two study groups, a comparison analysis of transcobalamin, homocysteine, HTC, and MMA levels was carried out using linear regression analysis for gender and age (Table 2).

To conclude the results, although there were significant differences in waist circumference, some vitals, and biochemical markers in both groups, vit. B12 and its metabolites levels showed no significant difference except for MMA (P < 0.001) (Table 2), keeping in mind that 138 subjects (117 patients and 21 controls) had missing values for this parameter.

Discussion

This study was carried out to investigate the impact of long-term metformin use on vit. B12 levels among T2DM patients. This study is, to the best of our knowledge, the first study in the Northern Emirates of the UAE to explore this healthcare concern. The mean diabetes illness duration in the patient cohort was 12 ± 6.8 years, and the metformin average dose was ≥ 2,000 mg daily. Our study findings revealed that T2DM patients using metformin had significantly higher levels of homocysteine than healthy controls. In addition, waist circumference and systolic blood pressure were higher in diabetes patients than in normal individuals, which is in line with other studies involving T2DM patients as part of the metabolic syndrome.

Out of 283 patients recruited with T2DM on metformin and 101 controls, we excluded 20 patients and 11 controls due to detection of high levels of transcobalamin (above 650 pmol/L) at the screening visit, which indicated the likelihood of undisclosed intake of vit. B12 supplements. Self-medication with multi-vit. supplementation, especially in chronic disease patients, is very common in the local population in the UAE.

Although our results showed a significant difference among both groups for homocysteine (P < 0.001), HTC (P < 0.01), and MMA (P < 0.001) levels, no significant difference was found (Chi-square analysis) in transcobalamin level.

As the protein responsible for the immediate availability of vit. B12, it is believed that measuring transcobalamin reflects the bioavailability of vit. B12. However, its significantly elevated levels among metformin users might indicate normal vit. B12 absorption, but on the other hand, vit. B12 level is considered low, as confirmed by the high level of homocysteine. Therefore, the surprisingly low level of MMA in both groups may necessitate further investigation and recruiting a larger number of participants in future studies.

The diagnostic precision of HTC remains debatable. The test is believed to have comparable sensitivities and specificities to serum vit. B12 when compared to MMA [29]. Several studies reported that HTC performed marginally better than the serum vit. B12 test [30]. Unfortunately, the HTC test specificity remains unclear. Moreover, homocysteine can be used as a test to determine the metabolic status of vit. B12. Recent studies reported that vit. B12 deficiency is mostly associated with elevated homocysteine levels, which is consistent with our study.

A panel of experts was advised to establish homocysteine thresholds based on age and folate supplementation status [31]. In folate-enhanced communities, the panel recommended 12 µmol/L for those aged 15 - 65 and 16 µmol/L for those aged > 65. For communities that do not implement folic acid

fortification, the recommended cutoffs are 15 and 20 µmol/L for those aged 15 - 65 years and > 65 years, respectively. The plasma homocysteine test has the same sensitivity as the MMA test. However, as we mentioned before, a complete set of these parameters should be available in future studies.

Conclusion

The current study revealed that T2DM patients using metformin had significantly higher levels of homocysteine compared to healthy controls, confirming the previously documented impact of metformin use on vit. B12 bioavailability. On the other hand, we also concluded that there is no significant difference between the two groups in either vit. B12 or its biomarker levels, with the exception of homocysteine, which is not an adequate variable to draw a solid conclusion. On the other hand, the sample for MMA analysis was too small to be considered; therefore, it was taken off consideration. However, the surprisingly high HTC and low MMA in the patient group warrants conducting more longitudinal multi-center studies to establish a meaningful relationship between metformin administration and vit. B12 deficiency.

Finally, the following recommendations are advisable for better control of metformin side effects. First, the monitored use of vit. B12 supplements in T2DM patients is very high, and this practice needs to be modified through patient education and awareness campaigns, both for patients and health care professionals. Second, local guidelines for vit. B12 screening and recommended follow-up investigations are highly needed to guide vitamin and supplementation prescribing.

Limitations

Sample size

Although the number recruited was enough to power the study, we had to exclude a number of subjects due to some missing data, which may have adversely affected the outcomes.

Age and gender

Age between test and control groups was not matched; vit. B12 levels tend to decline with age due to many factors, including absorption and dietary intake. In addition, gender in both groups was not matched; although T2DM is predominant in the local population, it was very difficult to find an adult over the age of 50 without diabetes, which may partially explain the younger age of the control group.

Concomitant medication administration

Medications, such as PPIs and H2 blockers that may interfere with vit. B12 absorption, are mostly prescribed for T2DM patients and could not be discontinued by our test group.

Undisclosed vit. B12 use

As vitamin supplementations are very common practice in the local population, a significant number of patients are on supplements without awareness, and it is very likely that some of the participants are taking vit. B12 supplements unintentionally without disclosure, which may have affected the results.

MMA data

MMA evaluation was not done on a significant number of patients, which may have affected the study results adversely. Future research studies should take into consideration the importance of measuring MMA as a sensitive indicator for vit. B12 deficiency.

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Conflict of Interest

The authors declare no conflict of interest.

Informed Consent

Written informed consents were obtained from all subjects involved before commencing the study.

Author Contributions

Conceptualization: MM and AE; patient recruitment/consenting: MM, YT, AE, GD, ME, and SS; methodology: MM, ME, YT, SK, and VK; software: SS; validation: MM, AE, GD, and YSM; formal analysis: SS; investigation: MM, AE, and YT; resources: MM and AE; data curation: MM, MAB, and AE; writing the original manuscript: MM, AE, ME, GD, and SS; reviewing and editing manuscript for submission: MM, YSM, MAB, and AE; visualization: SS, VK, YSM, and MAB; supervision: MM and AE; project administration: MM and VK. All authors have critically reviewed and approved

the final modified draft and are responsible for the content and similarity index of the manuscript.

Data Availability

The authors declare that data supporting the findings of this study are available within the article.

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