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## The Role of Metformin on Serum Vitamin B12 Levels in Patients with Type 2 Diabetes Mellitus

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### ABSTRACT

**Objectives.** To evaluate the vitamin B12 (VB12) deficiency in patients with type 2 diabetes mellitus (DM-2) using metformin or other hypoglycemic agents. **Methods.** 400 patients with DM-2 were divided into two arms (N = 200/group); 1) those receiving metformin (MET) for at least six months and 2) those receiving hypoglycemic agents other than metformin (OHA). Serum VB12 concentrations were measured. Data were analyzed by using two-sample t-test for numerical data and chi-square with logistic regression analysis for categorical data. Numerical values were expressed as means  $\pm$  standard deviations. Null hypotheses were rejected at  $P < 0.05$ . **Results.** Definite biochemical VB12 deficiency ( $<148$  pmol/L) was found in 29/200 (14.5%) of the MET group while it was observed in 4/200 (2%) in the OHA group ( $P < 0.001$ ). Similarly, possible VB12 deficiency (serum concentrations  $<185$  pmol/L) was found in 39 (19.2%) of the MET group and 12 (6%) in the OHA group ( $P < 0.001$ ). There was positive correlation between low VB12 level and metformin administration ( $R^2 = 0.26$ ,  $P < 0.001$ ). **Conclusions.** Patients with DM-2 on metformin had lower VB12 levels than those on other hypoglycemic drugs. The relation between VB12 deficiency and metformin therapy indicates the need for periodic measurement of serum VB12 levels in patients treated with metformin.

*Eur Res J 2015;1(1):1-7.*

**Keywords:** Oral medications, type-2 diabetes, metabolic syndrome, HbA1C, diabetic neuropathy

### Introduction

The care for diabetic patients is a complex process and involves managing disease complications, drug related side effects in

addition to glycemic control. There is supporting evidence that various interventions improve clinical outcome in patients with this disabling

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Received: 28.12.2014; Accepted: 16.01.2015; Published Online: 04.03.2015

disease [1]. Metformin is increasingly considered as an important component of the therapeutic standards in patients with type-2 diabetes mellitus (DM-2) [2]. It is usually well tolerated, although it may be associated with some gastrointestinal discomfort [2]. The American Diabetes Association (ADA) recommends metformin and lifestyle modification as the first line therapies for DM-2 [1]. Metformin is one of the few oral hypoglycemic agents that have been shown to improve cardiovascular morbidity and mortality among diabetic patients [3,4]. The unique effect of metformin on restoring sensitivity to insulin has greatly improved the prognosis of diabetic patients and provided added protection against vascular complications [5]. Previous studies suggest a higher prevalence of vitamin B12 (VB12) deficiency in diabetic patients treated with metformin [6, 8]. Although the association between metformin and VB12 deficiency has been widely described, little is known about its underlying pathophysiology. VB12, aka cobalamin, is a water-soluble vitamin that is essential for the normal function of the nervous system and erythropoiesis, through its required role in DNA synthesis. VB12 is essential for three enzymatic processes including the conversion of: a) homocysteine to methionine, b) methylmalonic acid to succinyl coenzyme A, and c) 5-methyltetrahydrofolate to tetrahydrofolate [9,10]. VB12 deficiency manifest itself with hematologic findings in the form of, macrocytic (megaloblastic) anemia and, in advanced cases, pancytopenia. Clinical signs and symptoms are divergent and go beyond the hematologic system. Nervous system is commonly affected in VB12 deficiency and causes varying degrees of sensory neuropathy progressing to combined sclerosis of the spinal cord in severe cases [11,13]. From a clinical standpoint, identification and prevention of metformin-related VB12 deficiency is the key to providing better care for diabetic patients [14].

In this study our primary aim was to define the prevalence of VB12 deficiency in DM-2 patients treated with metformin, and compare them to those patients receiving other hypoglycemic agents. Our primary endpoint was the occurrence

of serum VB12 concentrations below 148 pmol/L. We hypothesized that the prevalence of cobalamin deficiency is higher in patients receiving metformin as a part of their oral hypoglycemic regimen. The secondary aim of this study was to assess for confounding factors that may contribute to this vitamin deficiency.

## Materials and methods

The protocol and study design were reviewed and approved by the institutional review board of Tabriz University of Medical Sciences and its affiliated hospitals for its scientific merit and ethical consideration. The study design was cross-sectional and descriptive in nature. All study subjects were recruited from the pool of DM-2 patients presented to Tabriz University Endocrinology Clinic from January 2011 to March 2013. Following a careful screening for inclusion and exclusion criteria, the subjects were approached by one of the study team members and an informed consent was obtained. The enrolled patients were divided in two groups: 1) those receiving metformin for at least the past six months (MET; N=200); and 2) those receiving hypoglycemic agents other than metformin (OHA; N=200). Out of 200 patients in OHA group, 153 patients were on insulin alone; 12 were on insulin plus pioglitazone hydrochloride; 17 were on glibenclamide and 18 patients were on combination therapy with glibenclamide and pioglitazone hydrochloride.

Subject number and the related power was calculated by MedCalc Software® (Ostend, Belgium) based on the previously published serum VB12 concentrations in DM-2 patients on metformin. Considering a 7% difference in the prevalence of VB12 deficiency reported previously and accepting an alpha error of 0.05, inclusion of 400 patients yielded a power of 80% for this study.

Inclusion criteria were all type-2 diabetic patients within the age range of 18 to 65 years old who were on metformin minimum daily dose of 1 gram for past 6 months for the MET group, and no history of metformin use in the past five years

**Table 1.** Basic parameters between two studied groups

	<b>MET N=200</b>	<b>OHA N=200</b>	<b>P-Value</b>
Age (years)	52.2 ± 7.3	51.6 ± 10.0	0,78
Gender Male/Female	63/137 (31.5%)	68/132 (34%)	0,67
Weight (kg)	77.7 ± 13.1	76.3 ± 8.0	0,3
Height (m)	1.62 ± 1.09	1.62 ± 0.08	0,3
Body Mass Index (kg/m <sup>2</sup> )	29.7 ± 4.7	28.9 ± 3.7	0,11
Duration of the disease (years)	7 77 ± 5 56	8 12 ± 7 57	0 12

MET: metformin, OHA: oral hypoglycemic agent

for the OHA group.

Exclusion criteria were patients older than 65 years, alcoholism or drug abuse, known cases of malabsorption (gastrointestinal surgery, inflammatory bowel diseases and gluten allergy), chronic kidney disease with eGFR<30 mL/min (Stages IV and V), pernicious anemia, history of thyroid disease and thyroxin treatment and/or a history of other organ-specific autoimmune conditions (vitiligo, Addison's, primary ovarian failure, hypoparathyroidism), consumption of VB12 supplementation during the last three months, and receiving antibiotics or any medications known to influence gastrointestinal motility.

The demographic and related clinical data were collected by a study team member and recorded in Microsoft Excel worksheet. Comprehensive medication history was also documented. Fasting venous blood was obtained and blood samples were analyzed for complete blood cell count, comprehensive metabolic panel by the reference laboratory. Serum samples were then analyzed for VB12 concentrations by the same reference laboratory. This laboratory utilizes the Siemens Dimension Vista® system for VB12 assays. This system is based on a competitive immunoassay using direct chemiluminescent technology in which VB12 from the patient sample competes with VB12 labeled with acridinium ester. The system reports Vb12 results in pg/mL (mass units) and then converted to pmol/L (SI units). The conversion formula is 1 pg/mL = 0.74 pmol/L. The reportable range of this assay is 45 pg/mL (33 pmol/L) to 2000 pg/mL (1476 pmol/L). There is no definite consensus about the cut-off point of

VB12 deficiency, mainly because of diversity in studied populations and applied assay kits [15]. In this study, we defined deficiency as serum VB12 level of less than 148 pmol/L (200 pg/mL) [16]. Statistical analysis was performed using SPSS software package version 16.0 for windows. Numerical data were presented as mean ± standard deviation, while categorical variables were demonstrated as frequency and percentage (%). Categorical variables were compared by chi square test with Fisher's exact test while numerical variables were compared using independent samples t test. The correlations between variables were assessed by logistic regression analysis and the correlation coefficient values were reported. Null hypotheses were rejected when the P values were less than 0.05.

## Results

Out of 400 patients 131 (32.8%) were male and 269 (67.2%) were female. The average age for all patients was 51.9 ± 7.1 years old (range: 26-65 years). The average duration of disease in our series was 7.9 ± 5.2 years. There was no difference in age, body mass index and gender distribution between the MET and the OHA groups (Table 1). The average dose for metformin was 1255 ± 21 mg/day in the MET group. Serum concentrations of VB12 were 320 ± 18 pmol/L in the MET group, which were significantly lower than those of 410 ± 24 pmol/L in the OHA group (P<0.001). VB12 deficiency as defined by serum concentrations below 148 pmol/L was recognized in 33 out 400 patients (8.2%). VB12 deficiency

**Table 2.** Comparison of blood sugar, lipid profile, and blood markers between two studied groups

	<b>MET</b> <b>N=200</b>	<b>OHA</b> <b>N=200</b>	<b>P-Value</b>
Fasting Blood Sugar (mg/dL)	151.5 ± 50.5	159.2 ± 69.2	0.76
Hemoglobin A <sub>1</sub> C (%)	7.8 ± 1.3	8.2 ± 1.6	0.18
Triglycerides (mg/dL)	166 ± 73	154 ± 68	0.12
Cholesterol (mg/dL)	176 ± 64	178 ± 40	0.26
High Density Lipoproteins (mg/dL)	45.8 ± 11.6	43.8 ± 10.2	0.17
Low Density Lipoproteins (mg/dL)	98.2 ± 29.4	101.2 ± 28.7	0.12
Hemoglobin (g/dL)	14.5 ± 6.5	14.3 ± 5.9	0.69
Hematocrit (%)	42.1 ± 5.6	42.4 ± 4.5	0.57
Mean corpuscular volume (fL/RBC)	88.2 ± 5.2	88.9 ± 5.2	0.09
Serum Creatinine (mg/dL)	0.98 ± 0.20	1.06 ± 0.30	0.32

MET: metformin, OHA: oral hypoglycemic agent

## Discussion

was found in 29 patients (14.5%) of the MET group and 4 patients (2%) in the OHA group ( $P < 0.001$ ). There is a positive correlation between low VB12 levels and the use of metformin ( $R^2 = 0.26$ ,  $P = 0.001$ ). However, there was no significant correlation between VB12 levels and metformin dose ( $P = 0.711$ ).

Fasting serum glucose concentrations, as well as an indicator of long-term glucose control, hemoglobin A<sub>1c</sub> (HbA<sub>1c</sub>), was similar in the MET group compared to the OHA group (Table II). Similarly, there was no significant difference between the two treatment groups in their lipid profile, serum creatinine concentrations and hematologic profile (Table 2).

Additionally, there was no correlation between serum concentrations of VB12 and blood percentages of HbA<sub>1c</sub> in either study population. Spearman rank coefficient for this correlation was 0.091. Serum level for HbA<sub>1c</sub> was  $7.8 \pm 1.1$  % in patients with definite VB12 deficiency, which was comparable to  $8.3 \pm 2.0$  % in patients with possible VB12 deficiency and to  $8.0 \pm 1.5$  % in patients with normal serum VB12 ( $P = 0.45$ ).

In this study, we evaluated and compared the prevalence of VB12 deficiency in 400 patients with DM-2 using metformin or other hypoglycemic agents. Our study confirmed the finding of previous studies that metformin decreased serum VB12 levels and was associated with VB12 deficiency [14, 17-19]. Similarly, a randomized placebo-controlled trial of metformin for a period of 4 months on 390 patients with DM-2, reduced serum folate and VB12 level [19]. Therefore, the measurement of serum VB12 level may offer clinical guidance in managing diabetic patients on metformin therapy.

A study in a military primary care clinic estimated the prevalence of VB12 deficiency of 22% of the type 2 diabetic populations. They concluded that VB12 deficiency should be considered in DM-2 patients taking metformin, and recommended daily multivitamin in take to prevent VB12 deficiency [20]. VB12 deficiency has been defined by low serum levels of this vitamin ( $< 148$  pmol/L) in our series [16]. This is widely accepted serum concentration that is



considered as VB12 deficiency although its clinical presentation and severity vary from one patient to another [20]. Despite of lower serum concentrations of VB12, we did not encounter any clinical manifestations of vitamin deficiency in our series. Although we were not able to screen the patients for every subtle and non typical symptom of VB12 deficiency such as fatigue, memory loss, the question whether serum VB12 is an appropriate surrogate for the clinical diagnosis is still debatable. Additionally, there is significant overlap in neurologic symptoms of VB12 deficiency and diabetes that limits the specificity of these symptoms. Most of the studies previously have reported a 14-30% decrease in serum VB12 concentrations by oral metformin in diabetic patients. These authors have proposed a reduction in VB12 absorption in patients taking metformin for 6 months as the cause of VB12 deficiency [19, 21]. VB12 is released from animal proteins after being exposed to gastric acidity. After combining with intrinsic factor (IF) produced by gastric parietal cells, IF-B12 complex is absorbed in the terminal ileum [10].

Metformin induces VB12 malabsorption, which may increase the risk of developing VB12 deficiency [14, 17]. It is speculated that metformin may deteriorate cobalamin absorption by decreasing the reabsorption of bile salts in the ileum [22]. Interestingly, these authors and others report a reversal in VB12 malabsorption by increasing oral calcium intake that hastens the absorption of IF-B12 complex from the ileal mucosa [21, 23]. This discovery may be considered in treating diabetic patients on metformin who presents with drops in serum VB12 concentrations.

Additionally, metformin alters bacterial flora through an effect on gastrointestinal motility resulting in bacterial overgrowth similar to blind loop syndrome, which will further lead to VB12 malabsorption [21]. States of vitamin B12 deficiency and malabsorption were reported with increasing age > 70 years, [24, 26] and severe cases hypothyroidism [27]. However, in our case mix, we excluded those older than 65 years and clinical cases of hypothyroidism. Exclusion of

these patients is the most probable explanation for lack of association of vitamin B12 deficiency with age in our data series. It has been reported that higher doses and longer treatment with metformin are risk factors for B12 deficiency [14, 20]. Ting et al compared 155 DM-2 patients with metformin-related VB12 deficiency as defined in this study (< 140 pmol/L) with 310 matched controls. The dose of metformin was the strongest independent predictor of VB12 deficiency. In this case control study of metformin-related VB12 deficiency, metformin dose and duration of treatment emerged as the most consistent risk factors of VB12 deficiency within a diabetic population [14]. In a cross-sectional study in DM-2 patients treated with metformin, the mean time on metformin treatment was 43.5 months and mean drug dose was 1779 mg/day. Patients taking metformin had significantly lower VB12, but no correlation was found between VB12 plasma levels and metformin treatment time or dosage [28]. Liu et al examined the records of 56 geriatric patients with DM-2 treated with metformin and compared to those of 78 patients who were treated by other means [29]. The mean serum VB12 level was lower in the metformin group by 100 pmol/L. Eight patients in the metformin group and only 3 patients in the nonmetformin group had severe VB12 deficiency (<100 pmol/L). These authors while established a dose dependent pattern for serum VB12 concentrations, they were unable to demonstrate that serum VB12 levels further decrease as the duration of metformin increases. In the study by Nervo et al, serum VB12 level were inversely associated with age and the duration of metformin therapy, and directly associated with the estimated VB12 intake [17]. However, the association of metformin dose with serum VB12 level was not significant. In current study, serum VB12 levels were not associated with the dose of metformin. However, we could not make any comment about the duration of metformin therapy, as this variable was not recorded. Epidemiological studies have shown that the risks of both DM-2 and VB12 deficiency increase with age [18, 30]. The prevalence [ of VB12 deficiency has been reported to range from 20% to 60% in

the elderly population which is significantly higher than that in younger individuals [31]. Again, in our series we did not find any correlation between age and serum VB12. This disagreement between our findings and those previously published might have been due to the inclusion criteria, which limited our patient population up to 65 years old. The strengths of this study include its population based sampling. Moreover, the presence of a control group makes it possible to compare prevalence of VB12 deficiency in a similar diabetic patients not taking metformin. However, this study has important limitations. We have used only the serum VB12 level to define vitamin deficiency and we have not measured serum methylmalonic acid and homocysteine levels, which are more sensitive indicators of VB12 status than serum VB12 levels. Additionally, we have not recorded the amount of non-supplemental VB12 ingestion and therefore the daily intake of the VB12 from food resources has been ignored prior to blood sampling.

## Conclusions

Metformin therapy is associated with a higher prevalence of VB12 deficiency. This finding has implications for planning screening or prevention strategies in patients treated with metformin. As diabetic patients are prone to cardiovascular complication due to the microvascular nature of

their disease, VB12 deficiency adds to this risk by increasing serum homocysteine levels and accelerating the atherosclerotic processes. Although there is no evidence whether VB12 deficiency enhances the severity and the extent of diabetic neuropathy, it is conceivable to eliminate preventable factor as a possible cause of newly diagnosed neuropathies in these chronically ill patients.

The potential risk for developing VB12 deficiency highlights the necessity of checking serum VB12 concentrations during metformin therapy. We recommended using daily multivitamin to prevent VB12 deficiency in diabetic patients taking metformin. Among the patients with established VB12 deficiency (serum VB12 <150 pmol/L), supplemental VB12 should be administered either in the form of injection or orally. The role of daily calcium intake to reverse VB12 malabsorption needs to be further reinvestigated prior to its clinical use.

## Acknowledgement

The study was financially supported by the institutional funds From Tabriz University of Medical Sciences Research Foundation.

## Conflict of Interest Statement

None of the authors has any conflicts of interest

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