

Vitamin B₁₂ levels in metformin-treated type 2 diabetes patients



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INTRODUCTION

Metformin is a first-line drug in the pharmacotherapy of type 2 diabetes mellitus (DM). Apart from the low cost, good efficacy, and beneficial effects on body weight, the relatively safe adverse effect profile has justified the widespread use of metformin [1, 2]. Several studies have reported association of vitamin B₁₂ deficiency in type 2 diabetes patients treated with metformin [3—5]. Metformin does, however, induce vitamin B₁₂ malabsorption, which may increase the risk of developing vitamin B₁₂ deficiency — a clinically important and treatable condition. In addition, metformin treatment has been reported to be associated with decreased folate concentration, although the mechanism of this effect has not been elucidated. Finally, decreases in both folate and vitamin B₁₂ concentrations might, in turn, result in an increase in homocysteine concentrations, an independent risk factor for cardiovascular disease, especially among individuals with type 2 DM [6, 7].

All current evidence on vitamin B₁₂ deficiency in metformin treatment comes from short term studies [8—10]. No long term, placebo controlled data on the effects of metformin on concentrations of vitamin B₁₂ in patients with type 2 DM have been reported. In addition, placebo controlled data on the effects of metformin on homocysteine concentrations in type 2 diabetes are sparse, and again no long term data are available.

Clinically, vitamin B₁₂ deficiency could lead to altered mental status, megaloblastic anemia, and neurological

damage [11]. Peripheral neuropathy due to vitamin B₁₂ deficiency may be confused with diabetic peripheral neuropathy or may contribute to the aggravation of diabetic peripheral neuropathy [12]. The progression of neurologic damage due to vitamin B₁₂ deficiency can be stopped by early detection and treatment with cobalamin supplementation [13]. However, if this occurrence is misdiagnosed as diabetic neuropathy, permanent neurological damage may occur [14]. This study was done to further explore this question.

Aim of the study — to study the prevalence of vitamin B₁₂ deficiency and the factors associated with it in patients with type 2 diabetes mellitus who were treated with metformin.

MATERIALS AND METHODS

The patients were recruited from the endocrinology outpatient department of a tertiary care center from August 2018 to December 2018, after taking informed consent.

Patients were defined as type 2 DM with an age at diagnosis above 30 years. Pregnancy, pernicious anemia, malabsorption syndrome, gastrointestinal surgery, autoimmune thyroid disease, chronic hepatitis, chronic kidney disease stage 4 and above, chronic alcohol abuse, and the use of oral/parenteral vitamin B₁₂ or multivitamin supplements were exclusion criteria.

An observational study was conducted from August 2018 to December 2018. The study was approved by the University Ethical Committee.

Table 1
Baseline characteristics of patients with type 2 DM

Variable	Group 1, n = 35	Group 2, n = 25	P
Age, years	52.7 ± 3.4	49.4 ± 3.1	0.47
Sex, n (%)			
Male	16 (45.7)	11 (44.0)	0.34
Female	19 (54.3)	14 (56.0)	
Duration of DM, years	6.7 ± 1.1	1.9 ± 0.6	< 0.01
BMI, kg/m ²	29.3 ± 2.4	29.3 ± 2.4	0.23
HbA1c, %	7.9 ± 1.5	7.8 ± 1.4	0.30

DM — diabetes mellitus, BMI — body mass index,
HbA1c — glycated hemoglobin

A total of 53 patients with type 2 DM (group 1, n = 35, receiving metformin and group 2, n = 25, never treated with metformin) from the endocrinology clinic in Chernivtsi were studied.

Group 1 consisted of patients with type 2 DM with ongoing treatment with metformin with duration of metformin use > 6 months while second group consisted of patients with type 2 DM who had never received metformin.

History about diabetes onset, dose, and duration of metformin usage as well as recent (within 3 months) glycated hemoglobin (HbA1c) was obtained from Centre records. The Centre records, patient's prescriptions, and medicines were also searched for prescription of any vitamin B₁₂-containing supplements and patients were shown a list of commonly available multivitamins containing vitamin B₁₂ and were asked about their use at any time in the past.

Vitamin B₁₂ estimation was done by competitive chemiluminescent enzyme immunoassay on Immulite analyzer using commercial kits from Siemens Healthcare Diagnostics Inc., New York, USA. The calibration range of this assay was 197—771 pg/ml. Vitamin B₁₂ deficiency was defined as levels below 190 pg/ml.

Statistical analysis was performed using Statistical Package for Social Sciences SPSS version 14 (SPSS Inc., Chicago, IL, USA). Continuous variables were described as mean and standard deviation. The categorical variables were stated as proportions or percentages. The comparison between the first group and second group was done with Student's t-test for continuous variables and Chi-square test for categorical variables. Linear regression analyses were carried out to study the effect of duration since the diagnosis of diabetes, use of metformin, and duration of metformin use on

serum vitamin B₁₂ levels. P < 0.05 was considered statistically significant.

RESULTS

A total of 60 patients with type 2 DM (group 1, n = 35, receiving metformin and group 2, n = 25, never treated with metformin) from the endocrinology clinic in Chernivtsi were studied. Serum Vitamin B₁₂ levels were measured in all patients. The mean age of the study population was 51.9 ± 9.3 years. Table 1 shows the baseline characteristics of patients with type 2 DM. The two groups were comparable except for duration of DM which was significantly greater in the first group. Duration of metformin use was 26.2 ± 5.4 months (range 6—140 months). Daily dose of metformin was 839.2 ± 53.1 mg (range 500—2500 mg). The cumulative dose of metformin was 970.8 ± 517.2 g (range 85—10,590 g).

The serum mean unadjusted vitamin B₁₂ levels were 239.6 ± 37.4 pg/ml in the first group and 293.6 ± 42.3 pg/ml in the second group (p = 0.37).

The mean duration of DM was longer in the first group as compared to the second group (6.7 ± 1.1 years vs. 1.9 ± 0.6 years, p < 0.01). Vitamin B₁₂ deficiency was present in 16 (45.7 %) of the first group and in 6 (24.0 %) of the second group (p = 0.04).

On univariate linear regression analysis with vitamin B₁₂ levels as the dependent variable and metformin use (no and yes) as the predictor variable, metformin use was associated with a 8.7 ± 3.9 pg/ml (p = 0.73) lower vitamin B₁₂ level. On univariate linear regression analysis with vitamin B₁₂ levels as the dependent variable and duration of DM as the predictor variable, vitamin B₁₂ levels were 12.2 ± 3.0 pg/ml (95 % CI 6.4—18.0, p < 0.001) higher for every 1 year increase in the duration of DM.

To further study the association of vitamin B₁₂ levels and duration of DM, we performed a stratified analysis. Figure 1 shows the box plot of serum vitamin B₁₂ with duration of DM categorized into newly diagnosed (0—1 year), 1—5 years, and > 5 years. Serum vitamin B₁₂ levels were higher by 41.4 pg/ml in patients with DM of 1—5 years compared to those with recently diagnosed diabetes (p = 0.41). Serum vitamin B₁₂ levels were higher by 119.4 pg/ml in patients with duration of DM > 5 years compared to those with recently diagnosed diabetes (p < 0.02). Similarly, serum vitamin B₁₂ levels were 77.1 pg/ml higher in > 5 years DM duration group compared to 1—5 year duration of DM group (p = 0.03).

On univariate linear regression analysis with vitamin B₁₂ levels as the dependent variable and duration of

metformin use as the predictor variable, duration of metformin use predicted a 0.8 ± 0.4 pg/ml ($p = 0.05$) lower Vitamin B₁₂ levels for every 1 month increase in the duration of metformin use. On stratifying duration of metformin use into no metformin use, 0—1 years, 1—5 years, and more than 5 years, it was found that a 20.1 pg/ml ($p = 0.64$) and 37.3 pg/ml lower serum vitamin B₁₂ concentration was observed in individuals with a 0—1 years and 1—5 year duration of metformin use, respectively, compared with the group which had not received metformin.

In contrast, the serum concentration of vitamin B₁₂ was higher by 45.4 pg/ml ($p = 0.27$) in individuals who had received metformin for more than 5 years compared to those who had never received metformin. To understand the interplay of duration of DM and metformin use on serum Vitamin B₁₂ levels, a stratified analysis was carried out. A multivariate linear regression analysis with serum vitamin B₁₂ levels as the dependent variable and metformin use (no/yes) and duration of DM (stratified as 1—5 years and > 5 years) as predictor variables were done to adjust for the duration of DM. In this analysis, metformin use group was associated with a 87.3 ± 37.1 pg/ml ($p = 0.03$) lower serum vitamin B₁₂ levels. The serum vitamin B₁₂ levels were 104.9 ± 42.0 pg/ml ($p = 0.02$) higher in the 1—5 year duration of DM group while they were 192.3 ± 48.9 pg/ml ($p < 0.01$) higher in > 5 year duration of DM group.

DISCUSSION

The present study involving 60 patients with type 2 DM (35 metformin and 25 without metformin) showed lower vitamin B₁₂ levels with metformin use, when adjusted for duration since diagnosis of diabetes, which is consistent with other studies published earlier [1—7]. Without adjusting for duration of DM, there was neither a significant difference in serum vitamin B₁₂ levels nor in the prevalence of vitamin B₁₂ deficiency. However, the prevalence of vitamin B₁₂ deficiency in patients on metformin in our study is higher than that reported in literature [4—6, 15].

An interesting finding from our study is the statistically significant rise in vitamin B₁₂ with increasing duration of DM. In a cross-sectional study from the US comprising 1621 patients with type 2 DM (575 on metformin and 1046 not on metformin) and 6867 persons without diabetes, the DM without metformin group had the lowest prevalence of vitamin B₁₂ deficiency (2.4 %) as compared to 5.8 % in the diabetes on metformin group and the 3.3 % in the group without

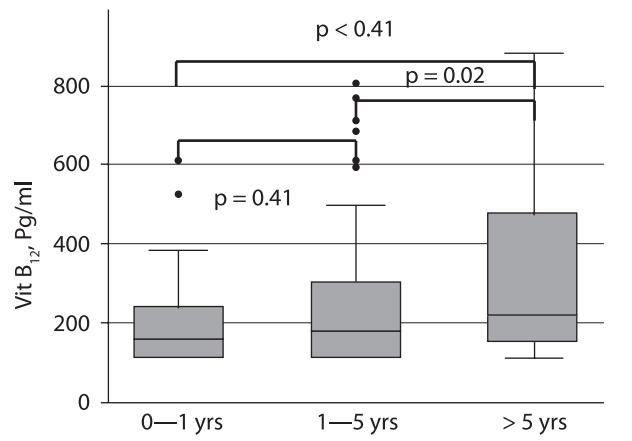


Figure 1. Vitamin B₁₂ levels and duration of type 2 DM (0—1, 0—5 years and > 5 years)

diabetes [3]. We did make an active effort to exclude patients who had been given vitamin B₁₂-containing supplements for any indication (review of available medical records was done, and patients were asked about the use of vitamin B₁₂-containing multivitamin supplements), but these preparations are available over the counter, and we cannot be sure that patients had never taken these medications earlier. In addition, the higher vitamin B₁₂ levels with greater duration of DM were seen in the second group also who were not at the risk of metformin-related vitamin B₁₂ deficiency (as they never received metformin) and hence are less likely to have received vitamin B₁₂ treatment.

The limitation of our study is that we did not measure functional markers of vitamin B₁₂ deficiency (serum homocysteine and serum methylmalonic acid levels) which may better reflect the status of vitamin B₁₂ levels in the body as compared to serum vitamin B₁₂ levels. In addition, our sample size was small. The strength of this study is that the second group included only those patients who had never taken metformin. The reason for choosing such a group was to exclude a possible long-term effect of metformin use on vitamin B₁₂ status. Vitamin B₁₂ is stored in the liver and several years may pass before the stores are depleted and detectable vitamin B₁₂ deficiency manifests. Thus, including patients with a history of metformin use in the no metformin group is a potential confounder which we excluded in this study.

CONCLUSIONS

Metformin use was associated with a significantly lower serum vitamin B₁₂ levels when adjusted for duration of diabetes.

Serum vitamin B₁₂ levels were higher by 41.4 pg/ml in patients with DM of 1—5 years compared to those with recently diagnosed diabetes (p = 0.41). Serum vitamin B₁₂ levels were higher by 119.4 pg/ml in patients with duration of DM > 5 years compared to those with recently diagnosed diabetes (p < 0.02). Similarly, serum vitamin B₁₂ levels were 77.1 pg/ml higher in > 5 years DM duration group compared to 1—5 year duration of DM group (p = 0.03).

Further study of the impact of duration of diabetes on serum vitamin B₁₂ levels and of functional markers of vitamin B₁₂ deficiency on hematological and neurological parameters will be interesting.

Джерела фінансування — відсутні. Гонорар за написання рукопису від комерційних організацій чи інших зацікавлених сторін автор не отримував.

Конфлікт інтересів — відсутній.

Етичні аспекти. Дослідження розглянуто Комітетом з етики ВДНЗУ «Буковинський державний медичний університет» (протокол № 8 від 17.09.2018 р.).

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ABSTRACT

Vitamin B₁₂ levels in metformin-treated type 2 diabetes patients

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Background. Metformin is the most widely used oral antihyperglycaemic drug, but it may lower B₁₂ status, which could have important clinical implications. There are limited data about the effect of metformin use on serum vitamin B₁₂ levels in type 2 diabetes mellitus (DM) patients.

Aim. To study serum Vitamin B₁₂ levels in patients with type 2 diabetes mellitus who were receiving metformin and compared them to those never treated with metformin.

Materials and methods. A total of 60 patients with type 2 DM (group 1, n = 35, receiving metformin and group 2, n = 25, never treated with metformin) from the endocrinology clinic in Chernivtsi were studied. Serum vitamin B₁₂ levels were measured in all patients.

Results and discussion. The serum vitamin B₁₂ levels were 239.6 ± 37.4 pg/ml in metformin group and 293.6 ± 42.3 pg/ml in the no metformin group (p = 0.37). When adjusted for duration of DM, metformin use was associated with a 57.2 ± 7.3 pg/ml (p = 0.03) lower serum vitamin B₁₂ levels. Serum vitamin B₁₂ levels were higher by 41.4 pg/ml in patients with DM of 1—5 years compared to those with recently diagnosed diabetes (p = 0.41). Serum vitamin B₁₂ levels were higher by 119.4 pg/ml in patients with duration of DM > 5 years compared to those with recently diagnosed diabetes (p < 0.02). Similarly, serum vitamin B₁₂ levels were 77.1 pg/ml higher in > 5 years DM duration group compared to 1—5 year duration of DM group (p = 0.03). Serum vitamin B₁₂ levels for the entire cohort were higher by 11.8 ± 1.7 pg/ml (p < 0.01) for every 1 year increase in the DM duration.

Conclusions. Metformin use was associated with a lower serum vitamin B₁₂ levels when adjusted for duration of DM. Increasing duration of DM was associated with higher serum vitamin B₁₂ levels.

Key words: type 2 diabetes mellitus, metformin, vitamin B₁₂ levels.

РЕЗЮМЕ

Вміст вітаміну B₁₂ у хворих на цукровий діабет 2-го типу

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Вступ. Метформін залишається найбільш використовуваним пероральним антигіперглікемічним препаратом, однак при цьому він може знижувати рівень вітаміну B₁₂, що може мати важливі клінічні наслідки. Наявні обмежені дані про вплив застосування метформіну на рівень вітаміну B₁₂ у сироватці крові у хворих на цукровий діабет 2-го типу (ЦД2).

Мета роботи — вивчити рівень вітаміну B₁₂ у сироватці крові у пацієнтів із цукровим діабетом 2 типу, які отримували метформін, та порівняти їх із пацієнтами, які ніколи не лікувалися метформіном.

Матеріали та методи. Під спостереженням перебували 60 пацієнтів із ЦД2 (група 1, n = 35, які отримували метформін, та група 2, n = 25, які ніколи не лікувалися метформіном) з Чернівецького обласного ендокринологічного центру. Рівень вітаміну B₁₂ у сироватці крові вимірювали у всіх пацієнтів. Результати. Рівень вітаміну B₁₂ у сироватці крові становив (239,6 ± 37,4) пг/мл у першій групі та (293,6 ± 42,3) пг/мл у другій групі (p = 0,37). Після скоригування за тривалістю ЦД2, використання метформіну було пов'язано з нижчим рівнем вітаміну B₁₂ у сироватці крові на (57,2 ± 7,3) пг/мл (p = 0,03). Рівень вітаміну B₁₂ у сироватці крові був більшим на 41,4 пг/мл у пацієнтів із тривалістю ЦД2 від одного до п'яти років порівняно з тими, у кого діабет був нещодавно діагностований (p = 0,41). Рівень вітаміну B₁₂ у сироватці крові був більшим на 119,4 пг/мл у пацієнтів із тривалістю ЦД2 > 5 років порівняно з пацієнтами з недавно діагностованим діабетом (p < 0,02). Аналогічно, рівень вітаміну B₁₂ у сироватці крові був на 77,1 пг/мл вище при тривалості ЦД2 понад 5 років порівняно з хворими з тривалістю ЦД2 1—5 років (p = 0,03). Рівень вітаміну B₁₂ у сироватці крові для всієї когорти був вищим на (11,8 ± 1,7) пг/мл (95 % ДІ 6,3—17,0, p < 0,01) за кожен рік збільшення тривалості ЦД2. Висновки. Застосування метформіну пов'язано з нижчим рівнем вітаміну B₁₂ у сироватці крові, що залежало від тривалості цукрового діабету.

Ключові слова: цукровий діабет 2-го типу, метформін, вітамін B₁₂.

РЕЗЮМЕ

Содержание витамина B₁₂ у больных сахарным диабетом 2-го типа

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Вступление. Метформин остается наиболее используемым пероральным антигипергликемическим препаратом, однако при этом он может снижать уровень витамина B₁₂, что может иметь важные клинические последствия. Имеются ограниченные данные о влиянии применения метформина на уровень витамина B₁₂ в сыворотке крови у больных сахарным диабетом 2-го типа (СД2).

Цель работы — изучить уровень витамина B₁₂ в сыворотке крови у пациентов с сахарным диабетом 2-го типа, получавших метформин, и сравнить

их с пациентами, которые никогда не лечились метформинном.

Материалы и методы. Под наблюдением находились 60 пациентов с СД2 (группа 1, $n = 35$, получавших метформин и группа 2, $n = 25$, которые никогда не лечились метформинном) из Черновицкого областного эндокринологического центра. Уровень витамина B_{12} в сыворотке крови измеряли у всех пациентов. Результаты. Уровень витамина B_{12} в сыворотке крови составил ($239,6 \pm 37,4$) пг/мл в первой группе и ($293,6 \pm 42,3$) пг/мл во второй группе ($p = 0,37$). После коррекции по продолжительности СД2 использование метформина было связано с более низким уровнем витамина B_{12} в сыворотке крови на ($57,2 \pm 7,3$) пг/мл ($p = 0,03$). Уровень витамина B_{12} в сыворотке крови был больше на 41,4 пг/мл у пациентов с длительностью СД2 от одного до пяти лет по сравнению с теми,

у кого диабет был недавно диагностирован ($p = 0,41$). Уровень витамина B_{12} в сыворотке крови был больше на 119,4 пг/мл у пациентов с длительностью СД2 свыше 5 лет по сравнению с пациентами с недавно диагностированным диабетом ($p < 0,02$). Аналогично, уровень витамина B_{12} в сыворотке крови был на 77,1 пг/мл выше при продолжительности СД2 более 5 лет по сравнению с больными с длительностью СД2 1—5 лет ($p = 0,03$). Уровень витамина B_{12} в сыворотке крови для всей когорты был выше на ($11,8 \pm 1,7$) пг/мл (95 % ДИ 6,3—17,0, $p < 0,01$) на каждый год увеличения продолжительности СД2. Выводы. Применение метформина связано с низким уровнем витамина B_{12} в сыворотке крови, что зависело от продолжительности сахарного диабета.

Ключевые слова: сахарный диабет 2-го типа, метформин, витамин B_{12} .

Дата надходження до редакції 27.11.2019 р.