ARTIGO ORIGINAL

Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil

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Conflict of interest: None.

Summary

Objective: The objective of this study was to evaluate the presence of vitamin B12 deficiency and the factors associated with serum vitamin B12 levels in a sample of metformin-treated Brazilian diabetic patients. Method: Cross-sectional study. Results: 144 patients were included. Serum vitamin B12 levels were low (< 125 pmol/L) in 10 patients (6.9%) and possibly low (125 - 250 pmol/L) in 53 patients (36.8%). Serum vitamin B12 levels were negatively associated with age (B = -3.17; β = -0.171; p = 0.037) and duration of metformin use (B = -33.36; β = -0.161; p = 0.048), and positively associated with the estimated intake of vitamin B12 (B = 67.96; β = 0.249; p = 0.002). Conclusion: The present findings suggest a high prevalence of vitamin B12 deficiency in metformin-treated diabetic patients. Older patients, patients in long term treatment with metformin and low vitamin B12 intake are probably more prone to this deficiency.

Keywords: Diabetes mellitus; vitamin B 12; metformin.

Resumo

Vitamina B12 em pacientes diabéticos usando metformina: um corte transversal no Brasil

Objetivo: O objetivo deste estudo foi avaliar a prevalência de deficiência de vitamina B12 em pacientes diabéticos brasileiros tratados com metformina e os fatores associados aos níveis séricos de vitamina B12. Método: Corte transversal. Resultados: 144 pacientes foram incluídos. Os níveis séricos de vitamina B12 foram baixos (< 125 pmol/L) em 10 pacientes (6,9%) e possivelmente baixos (125 - 250 pmol/L) em 53 pacientes (36,8%). Os níveis séricos de vitamina B12 foram associados negativamente à idade (B = -3,17; β = -0,171; p = 0,037) e à duração do uso de metformina (B = -33,36; β = -0,161; p = 0,048) e positivamente com a ingestão estimada de vitamina B12 (B = 67,96; β = 0,249; p = 0,002). Conclusão: Estes resultados sugerem alta prevalência de deficiência de vitamina B12 em pacientes diabéticos tratados com metformina. Pacientes mais velhos, em uso de metformina há muito tempo e com ingestão baixa de vitamina B12 estão provavelmente mais predispostos a essa deficiência.

Unitermos: Diabetes mellitus; vitamina B 12; metformina.
**INTRODUCTION**

Vitamin B12 deficiency can lead to hematological abnormalities, cognitive disorders, and neuropathy. This vitamin is obtained from animal origin food, so its deficiency may be related to eating habits, changes in the absorption or metabolic mechanisms.

Metformin is the first choice of treatment for type 2 diabetes mellitus (DM2), decreasing insulin resistance, and cardiovascular and mortality risks. It is usually well tolerated, although it may cause gastrointestinal side-effects.

The association of metformin with vitamin B12 deficiency has been studied for some time, yet, the mechanism leading to this deficiency is not fully understood. Recently, vitamin B12 deficiency has been associated with neuropathy in metformin-treated diabetic patients.

A literature review was made in the PubMed and LILACS databases combining the keywords: vitamin B12 and metformin and no studies with Brazilian patients were identified until August, 2010. Given the importance of vitamin B12 deficiency, this study aimed to estimate its prevalence and to evaluate factors associated with serum vitamin B12 levels in metformin-treated DM2 patients, in the outpatient setting of a tertiary hospital in Southern Brazil.

**METHODS**

All DM2 patients treated with metformin for more than one year in the ambulatory of Internal Medicine at the Hospital de Clinicas de Porto Alegre were invited to participate, until completion of the sample. Patients with partial or total gastrectomy, Crohn’s disease, disorders of the terminal ileum and using supplemental vitamin B12 were excluded. Factors evaluated in the study were age, gender, weight, metformin dosage, duration of metformin use, dietary intake of vitamin B12, red cell mean corpuscular volume (MCV), glycosylated hemoglobin and use of other medications or supplements.

Sample size was set to 147 patients estimating a 25% prevalence of vitamin B12 deficiency, with an accuracy of 7%, and p < 0.05.

Intake of vitamin B12 was estimated by the 24-hour food recall method. The book *Álbum fotográfico de porções alimentares* (Photographic Record for Dietary Inquiries): *aplicances and parts* was used to illustrate the food portions. A table was used to convert approximate cooking measurements into grams. Based on this information, intake of vitamin B12 was estimated using a chart containing the amount of vitamin B12 in food in Brazil. Vitamin B12 intake was considered adequate or not according to the Dietary Reference Intake (DRI) for vitamin B12, as proposed by the Institute of Medicine of the USA.

This study was approved by the Ethics Committee on Research of the Hospital de Clinicas de Porto Alegre and all patients signed an informed consent before entering the study. One blood sample from each patient was obtained and serum samples were stored at -70°C. Vitamin B12 levels were determined in a single batch by electrochemiluminescence - Modular E170 Roche. Vitamin B12 deficiency was defined by a serum level < 125 pmol/L and was considered possible when the serum level of vitamin B12 ranged from 125 pmol/L to 250 pmol/L.

Data analysis was performed with the Statistical Package for the Social Sciences (SPSS) version 16.0. Association of serum vitamin B12 levels with the factors studied was evaluated by Spearman’s correlation test, Student’s t test or the Mann-Whitney test. All factors associated with the serum vitamin B12 levels with p ≤ 0.2 were included in a linear regression model to determine the factors independently associated, as suggested by Altman. The variables without normal distribution were transformed into their natural logarithms. Values of p < 0.05 were considered statistically significant.

**RESULTS**

In the period from July 3 to August 13, 2009, 158 eligible patients were identified and all agreed to participate. Two patients were excluded for reporting irregular use of metformin. Twelve patients were excluded for not undergoing blood collection. The analysis was done based on data obtained from 144 patients. Characteristics of these patients are shown in Table 1.

Serum vitamin B12 levels were low (< 125 pmol/L) in 10 patients (6.9%) and possibly low (125 pmol/L to 250 pmol/L) in 53 patients (36.8%) and were associated with age, estimated vitamin B12 intake and body weight, as shown in Figure 1. The association of metformin dose and duration of metformin use with serum vitamin B12 levels was, respectively, r = -0.114, p = 0.173 and r = -0.128, p = 0.129. Sixty eight patients (47%) had adequate daily intake of vitamin B12 (≥ 2.4 µg).

**Table 1 – Characteristics of metformin-treated diabetic patients (n = 144)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.7 ± 11.30</td>
</tr>
<tr>
<td>Women n (%)</td>
<td>91 (63.2)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>80.3 ± 15.4</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.62 ± 0.09</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.62 ± 5.18</td>
</tr>
<tr>
<td>Time using metformin (years)</td>
<td>4 (2.8.1)</td>
</tr>
<tr>
<td>Dose of metformin (mg)</td>
<td>2550 (1700/2550)</td>
</tr>
<tr>
<td>Use of omeprazole n(%)</td>
<td>57 (39.6)</td>
</tr>
<tr>
<td>Use of calcium carbonate n(%)</td>
<td>17 (11.8)</td>
</tr>
<tr>
<td>Hb A1c (%)</td>
<td>7.70 (6.88/9.73)</td>
</tr>
<tr>
<td>Red cell MCV (fL)</td>
<td>88.0 ± 4.6</td>
</tr>
<tr>
<td>Daily intake of B12 (µg)</td>
<td>2.25 (1.36/3.38)</td>
</tr>
<tr>
<td>Serum vitamin B12 (pmol/L)</td>
<td>311.0 ± 155.5</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation, median (25/75 percentile) or n (%).
Table 2 – Factors associated with serum vitamin B12 levels in metformin-treated type 2 diabetic patients (n = 144)

<table>
<thead>
<tr>
<th>Variables</th>
<th>B</th>
<th>SE</th>
<th>β</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated vitamin B12 intake</td>
<td>67.957</td>
<td>21.894</td>
<td>0.249</td>
<td>0.002</td>
</tr>
<tr>
<td>Age</td>
<td>-3.172</td>
<td>1.505</td>
<td>-0.171</td>
<td>0.037</td>
</tr>
<tr>
<td>Metformin use duration</td>
<td>-33.358</td>
<td>16.733</td>
<td>-0.161</td>
<td>0.048</td>
</tr>
</tbody>
</table>

*Model including vitamin B12 intake, age and metformin use duration, dependent variable: serum vitamin B12; adjusted $R^2 = 0.111$
Although serum vitamin B12 level is the most used test to diagnose deficiency of this vitamin due to its low cost, its sensitivity is not very high. It can be in the normal range in a significant proportion of patients with disabilities in which serum homocysteine or methylmalonic acid levels allow the early identification of vitamin B12 deficiency. The large number of metformin-treated DM2 patients at risk for vitamin B12 deficiency makes it important to have better tests to clearly diagnose this condition, prior to committing these patients to lifelong treatment with vitamin B12. Although our study did not answer several questions, we believe it is important to call the medical community’s attention to this common problem.

REFERENCES