Prevalence of Vitamin B_{12} Deficiency in Patients with type 2 Diabetes Mellitus on Metformin

Talar M Raqib (MBChB), Ranan K Polus (FIBMS, IFCAP)

\(^1\)Kurdistan Higher Council for Medical Specialties, Erbil, Iraq
\(^2\)Kurdistan Higher Council for Medical Specialties, Erbil, Iraq
\(^3\)Pathology Department, College of Medicine, Hawler Medical University, Erbil, Iraq

Abstract

**Background:** Type 2 diabetes mellitus (T2DM) is a common public health problem in metabolism. Metformin is the oral hypoglycemic agent used as a first line together with life style modification in type 2 diabetes patients worldwide. Continuous metformin therapy increases the risk of vitamin B12 insufficiency, and its medical consequences in T2DM patients.

**Objective:** To detect the prevalence of serum vitamin B_{12} deficiency in T2DM who has been treated with metformin in Erbil Province.

**Patients and Methods:** The study involved 200 cases (100 patients and 100 controls) that met the study’s basic criteria. A completed questionnaire, and a blood test for serum vitamin B_{12} levels were performed. A deficiency of vitamin B_{12} is defined as <160 pg/mL in serum vitamin B_{12}.

**Results:** Deficiency of serum vitamin B_{12} was found in 48% of patients (n=48), while HbA1c levels had no impact on this finding. In T2DM level of serum vitamin B_{12} that has been on metformin at a dose of ≤ 1 gm/ day shows a significant difference with those patients with no history of metformin use.

**Conclusion:** Low levels of serum vitamin B_{12} came as a result of the overdosing of metformin for long period of treatment.

**Keywords:** Type 2 diabetes mellitus, metformin, serum vitamin B_{12} deficiency

Introduction

Diabetes mellitus is a term used to describe a group of metabolic diseases that all have a hyperglycemic phenotype[1]. A complicated combination between heredity and environmental factors results in several clinically different kinds of diabetes mellitus. Reduced insulin secretion, decreased glucose utilization, and increased glucose production are all factors that contribute to hyperglycemia[2]. Several oral hypoglycemic medications are used to treat type 2 diabetes mellitus.

Metformin is the first-line oral hypoglycaemic medication because of its low cost, high efficacy, and weight-loss benefits. It is also often used as a dietary modification [2, 3]. Metformin mainly affects the liver by reducing the release of glucose and then increasing the intake of glucose in the peripheral tissues, especially muscles [4]. Metformin inhibits vitamin B_{12} absorption...
via changing intestinal motility, causing bacterial overgrowth, and changing the vitamin B₁₂-IF complex. The likelihood of developing metformin-induced vitamin B₁₂ insufficiency is influenced by the patient's age, metformin dosage, and treatment duration [5]. Metformin's side effects include nausea, upset stomach, lactic acidosis, and reduced absorption of vitamin B₁₂ [6].

Vitamin B₁₂, also known as cyanocobalamin, is a critical component found in animal diets. It is a necessary cofactor in the creation of deoxyribonucleic acid (DNA) and metabolic processes [5].

Vitamin B₁₂ is essential for brain, and neurological system function, as well as the creation of red blood cells. Peripheral neuropathy can be brought on by T₂DM patients who are vitamin B₁₂ deficient [7]. B₁₂ deficiency is common in patients taking long-term metformin therapy, with rates ranging from 5.8% to 30%.[8] Metformin prevents vitamin B₁₂ absorption in the small intestine (ileum) [9], and it causes block of calcium-dependent channels in the ileum [4, 10].

Many metformin users suffer from vitamin B₁₂ insufficiency[7], which is frequently ignored and rarely checked, resulting in paraesthesias and anemia that are mistakenly attributed to underlying DM by physicians and hence never addressed [11]. Vitamin B₁₂ is essential for the conversion of homocysteine (Hcy) to methionine, and a deficiency can lead to high homocysteinemia, which has been associated with macrovascular issues and may aggravate peripheral neuropathy in T₂DM patients [1]. Deficiency of vitamin B₁₂ usually appears in the form of haematological and neurological symptoms. Without a haematologic presentation, neurological symptoms may be the only sign of a deficiency [12, 13]. On the other hand, neurological manifestations come before hematological alterations [4].

In Iraq, there are large numbers of diabetes and its consequences; there is little study on vitamin B₁₂ deficiency associated with the use of metformin [14, 15]. As a first step in identifying vitamin B₁₂ deficiency in T₂DM patients, a serum vitamin B₁₂ level should be determined. It is usually indicated by concentrations of less than 200pg/mL [16, 17].

Patients and Methods
Design of the research
From April to August 2021, diabetes patients who visited the Layla Qasim diabetic center, Hawler teaching hospital, and Rizgary teaching hospital as outpatients were studied in this cross-sectional study. A total of 200 cases (100 patients and 100 controls) were targeted for the study. The samples were gathered after taking a short history from each patient. At the time of the vitamin B₁₂ measurement, information about age, gender, weight, height, diabetes mellitus as a medical condition, and metformin (a medication used to treat it; dosage and frequency of administration daily) were gathered. Our patients were split into two groups: 100 cases of diabetes patients who took metformin (Glucophage, 1000 mg tab, Merck Company) and other hypoglycemic drugs, and 100 cases of control patients.

Data collection
Four milliliters of venous blood were obtained using a full aseptic procedure following fasting overnight. Two milliliters of blood were distributed into EDTA tubes,
with the remaining two milliliters going into gel separator tubes. The sample in the EDTA tube was used to determine glycated hemoglobin (HbA1c) using a Cobas c 311 automated analyzer and complete blood counts (CBC) using a Mythic 18 automated analyzer. To get the serum, the gel separator tubes were placed in a centrifuge and spun at 3000 rpm for 5 minutes. Cobas e 411 was used to calculate serum vitamin B\textsubscript{12} levels (1). A pathologist then checked the results. The levels of serum vitamin B\textsubscript{12} less than 160pg/mL were considered deficient, whereas values of more than 160-220pg/mL and >220 were considered borderline and normal serum vitamin B\textsubscript{12}, respectively (17). The biodata, serum hemoglobin level, time of diabetes onset, metformin dosage, and duration of metformin use were all included in each patient's profile. The work sheet was created, and all of the information was saved. The study was approved by the Kurdistan Board of Medical Specialties.

### Statistical Analysis

On an excel sheet, all the information has been entered. Appropriate statistical analysis will be employed in order to analyze all data during the final analysis in order to provide an observer survey by Chi-square and ordinary one-way ANOVA. A P-value of less than 0.05 was considered statistically significant. The prism (graph pad (6.1)) was used in all of the analyses.

### Results

The important comparisons of characteristics between cases and controls with and without deficiency of vitamin B\textsubscript{12} are shown in Table (1). 100 patients with type 2 diabetes mellitus (30 males and 70 females) were recruited for the study, and 100 control cases (48 males and 52 females) were also included. There is a significant difference between patients and case control in serum vitamin B\textsubscript{12} deficiency (p<0.005). The highest rate of serum vitamin B\textsubscript{12} deficiency was found in females (34 cases) as compared with normal serum vitamin B\textsubscript{12} (7 cases) in male diabetic cases. There is a non-significant difference between them. The demographic data was used to determine age, weight, and body mass index (BMI) characteristics linked to serum vitamin B\textsubscript{12} deficiency as shown in Table (2).

**Table (1): Level of serum vitamin B\textsubscript{12} according to the gender from patients and control cases**

<table>
<thead>
<tr>
<th>Serum Vitamin B\textsubscript{12} level</th>
<th>Diabetic Case</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Patients</td>
<td>Male</td>
</tr>
<tr>
<td>&lt;160 (B\textsubscript{12} deficiency)</td>
<td>48</td>
<td>14 (14%)</td>
</tr>
<tr>
<td>160-220 (Border Line)</td>
<td>31</td>
<td>9 (9%)</td>
</tr>
<tr>
<td>&gt;220 (Normal Vitamin B\textsubscript{12})</td>
<td>21</td>
<td>7 (7%)</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>30</td>
</tr>
</tbody>
</table>

* P value=0.0001
Table (2): Serum vitamin B<sub>12</sub> level according age, weight and BMI

<table>
<thead>
<tr>
<th>Serum Vitamin B&lt;sub&gt;12&lt;/sub&gt; level</th>
<th>Total Patients</th>
<th>Age Mean±S.D</th>
<th>weight Mean±S.D</th>
<th>BMI Mean±S.D</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;160 (B&lt;sub&gt;12&lt;/sub&gt; deficiency)</td>
<td>48</td>
<td>52.96±7.82</td>
<td>83.54±10.9</td>
<td>30.41±4.42</td>
<td>0.1611</td>
</tr>
<tr>
<td>160-220 (Border Line)</td>
<td>31</td>
<td>51.74±9.90</td>
<td>75.97±16.04</td>
<td>28.26±5.41</td>
<td></td>
</tr>
<tr>
<td>&gt;220 (Normal Vitamin B&lt;sub&gt;12&lt;/sub&gt;)</td>
<td>21</td>
<td>56.43±9.08</td>
<td>77.24±11.29</td>
<td>28.27±4.54</td>
<td></td>
</tr>
</tbody>
</table>

Figure (1) showed that the level of serum vitamin B<sub>12</sub> is unrelated to fasting blood sugar and glycosylated hemoglobin (HbA1c). In the borderline of serum vitamin B<sub>12</sub> levels, according to T2DM, FBS and HbA1c were found to be (256.5±133.6) and (8.083±2.274), respectively.

Table (3): Level of Serum Vitamin B<sub>12</sub> according Fasting blood Sugar (FBS) and HbA1c

<table>
<thead>
<tr>
<th>Serum Vitamin B&lt;sub&gt;12&lt;/sub&gt; level</th>
<th>FBS Mean±S.D</th>
<th>HbA1c Mean±S.D</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;160 (B&lt;sub&gt;12&lt;/sub&gt; deficiency)</td>
<td>213.9±100.6</td>
<td>7.998±1.292</td>
</tr>
<tr>
<td>160-220 (Border Line)</td>
<td>256.5±133.6</td>
<td>8.083±2.274</td>
</tr>
<tr>
<td>&gt;220 (Normal Vitamin B&lt;sub&gt;12&lt;/sub&gt;)</td>
<td>194.5±86.52</td>
<td>7.671±1.991</td>
</tr>
</tbody>
</table>

Figure (2) shows the haematological parameters in T2DM patients with a level of serum vitamin B<sub>12</sub> which aimed to establish the blood marker most responsive to changes in vitamin B<sub>12</sub> concentration. Statistically, there is no significant difference between Hb, MCV, and MCH with serum vitamin B<sub>12</sub> levels.
Based on levels of serum vitamin B₁₂, all patients were separated into three groups during the duration of type 2 diabetic patients and metformin therapy, as indicated in Figure (3). Statistically, there is a significant difference between both groups (p < 0.001).

** Figure (2): Level of Serum Vitamin B₁₂ according Hemoglobin concentrations (Hb), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCH) **

** Figure (3): Level of Serum Vitamin B₁₂ in Relation to Various Factors (T₂DM Duration and Metformin Usage time) **
Deficiency of vitamin B\textsubscript{12} (<160 pg/mL) was higher significantly (p=0.0161) in users of metformin 37 (37%), as compared with other medication (glimepride tablets and insulin) 11 (11%), while normal vitamin B\textsubscript{12} (>220 pg/mL) was higher in users of metformin 11 cases, as compared with other medication users 10 cases Table (4). Patients who used more than 1000 mg of metformin per day had a lower level of serum vitamin B\textsubscript{12} than those who took less than 1000mg per day. According to Table (5), there is statistically a significant correlation between the dose of metformin and the range of serum vitamin B\textsubscript{12} levels in our study.

<p>| Table (4): Serum Level of Vitamin B\textsubscript{12} according Metformin and other Medication (glimepride tablets and insulin) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Level of Serum Vitamin B\textsubscript{12}</th>
<th>Total Patients</th>
<th>Metformin (n=75)</th>
<th>other O.H (n=25)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;160 (B\textsubscript{12} deficiency)</td>
<td>48 (48%)</td>
<td>37 (37%)</td>
<td>11 (11%)</td>
<td>0.0161</td>
</tr>
<tr>
<td>160-220 (Border Line)</td>
<td>31 (31%)</td>
<td>27 (27%)</td>
<td>4 (4%)</td>
<td></td>
</tr>
<tr>
<td>220 (Normal Vitamin B\textsubscript{12})</td>
<td>21 (21%)</td>
<td>11 (11%)</td>
<td>10 (10%)</td>
<td></td>
</tr>
</tbody>
</table>

<p>| Table (5): Characteristics of Level Serum Vitamin B\textsubscript{12} based on Metformin Dose |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Serum Vitamin B\textsubscript{12} level</th>
<th>Total</th>
<th>&gt;1000mg/day (%)</th>
<th>&lt;1000mg/day (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;160 (B\textsubscript{12} deficiency)</td>
<td>37</td>
<td>35 (46.67%)</td>
<td>2 (2.67%)</td>
<td>0.0009</td>
</tr>
<tr>
<td>160-220 (Border Line)</td>
<td>27</td>
<td>15 (20%)</td>
<td>12 (16%)</td>
<td></td>
</tr>
<tr>
<td>220 (Normal Vitamin B\textsubscript{12})</td>
<td>11</td>
<td>7 (9.33%)</td>
<td>4 (5.33%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>75</td>
<td>57 (76%)</td>
<td>18 (24%)</td>
<td></td>
</tr>
</tbody>
</table>

**Discussion**

Out of 100 diabetic patients, the highest rate of serum vitamin B\textsubscript{12} deficiency was found in females (70 cases) as compared with males (30 cases). These agreed with Yakubu et al., [10] and Krishnan et al., [18], who revealed that the lowest rate was found in males at 59 (30.1%) and 79 (38.5%) as compared to 137 (69.1%) and 126 (61.5%) females, respectively [10, 18]. When compared to male diabetic patients' normal serum vitamin B\textsubscript{12} levels (7%) in Table (1), female diabetic patients had the highest rate of vitamin B\textsubscript{12} deficiency (34%). This agrees with Jeetendra et al., [16], who have shown that, in patients with T\textsubscript{2}-DM, the level of serum vitamin B\textsubscript{12} should be used as an initial screening technique for diagnosing deficiency of vitamin B\textsubscript{12} [16]. Deficiency of vitamin B\textsubscript{12} is usually indicated by concentrations of less than 200 pg/ml, but concentrations greater than 220 pg/mL indicate the absence of vitamin B\textsubscript{12} deficiency. As well, Khan et al., [17] explain that the levels of vitamin B\textsubscript{12} in the blood ranged from 122 to 2034pg/mL.[17]. Furthermore, concurrent intake of metformin, which lowers stomach acidity and plays a key role in vitamin B\textsubscript{12} deficiency, and the parietal cells produce stomach acid, which is necessary for the absorption of vitamin B\textsubscript{12} from dietary sources, causes the parietal cells to produce less acid [15, 19].

Patients' demographic data was used to detect potential vitamin B\textsubscript{12} deficiency-related factors (age, weight, and BMI). There is no significant difference between levels of serum vitamin B\textsubscript{12} with these factors. These
agreed with Owhin et al., [2] and Krishnan et al., [18], which showed that, there was no significant relationship between age, sex, weight, height, and level of serum vitamin B₁₂ insufficiency. In numerous observational studies, serum vitamin B₁₂ insufficiency was found in 5.8% of T2DM patients over 50 who received metformin for a median of 5 years, compared to 2.4% of T2DM patients who did not receive the drug [1, 4, 10, 19]. Figure (1) showed that the level of serum vitamin B₁₂ is unrelated to fasting blood sugar and glycosylated hemoglobin (HbA₁c). Based on T₂DM, FBS and HbA₁c were discovered (256.5±133.6) and (8.083±2.274), respectively in the border line of serum vitamin B₁₂ Table (3). Keep in mind that HbA₁c values are linked to blood glucose levels[20]. In our results, we discovered that HbA₁c levels were not associated with vitamin B₁₂ as compared with Zhao et al., and Ko et al., [21, 22]. Metformin-induced vitamin B₁₂ insufficiency in T₂DM patients has been associated with changes in small intestine motility, bacterial flora, competitive inhibition, sluggish vitamin B₁₂ absorption, and alterations in intrinsic-factor levels. According to Carrizzo et al., [23] and Lata et al., [24], glycosylated hemoglobin levels in patients prescribed with metformin reduced mean HbA₁c levels by 1.1%, and a comparison between low dose and high dose metformin. HbA₁c levels were 0.3% lower with high dose therapy [23, 24]. The haematological parameters in T2DM patients with a level of serum vitamin B₁₂ are shown in Figure (2). Alternatively, a deficiency of vitamin B₁₂, as measured by the level of serum vitamin B₁₂, had no effect on the hematological parameters. Previously, similar findings have been reported [25]. However, total level of serum vitamin B₁₂ may not adequately reflect the body's Vitamin B₁₂ status; therefore biochemical shortage may not always equate to clinical deficiency [10]. Changes in small intestine motility and increased bacterial overgrowth have been linked to metformin-induced B₁₂ deficiency, as has metformin's interference with calcium-dependent intrinsic factor release [26]. The elevated incidence of anaemia in this group could be due to a number of reasons. One possible explanation is that our patients are older, as the prevalence of anemia has been found to be higher in older age groups. Our findings revealed that metformin therapy had a considerable impact on vitamin B₁₂ levels in T₂DM patients [11, 27, 28].could indicate a dietary shortage or be the result of metformin use. As well, metformin duration was the most consistent risk factor for vitamin B₁₂ deficiency [9, 13, 16]. According to mainstream recommendations, metformin is the first medicine of choice for T₂-DM. When there are contraindications to its usage or patients can’t take it due to bad effects, physicians have a choice of additional classes of drugs to treat hyperglycemia linked with T₂DM. Each type of agent has its own set of advantages and risks. When choosing another to metformin, there are a number of factors such as overall efficacy in lowering HbA₁c, adverse effect profile, cost, and patient preference. The number of factors that influence the decision-making process is often difficult to determine, and no single specific agent is ideal [29, 30]. On the other hand, Table (5) shows that patients who took more than 1000 mg of metformin per day had a lower level of
serum vitamin B₁₂ than those who took less than 1000 mg, as similar results have been discovered [1, 16, 30]. Although the specific mechanism of deficiency of vitamin B₁₂ caused by a high dose of metformin is still unknown, in patients using high doses of metformin, we observed a stronger reduction of vitamin B₁₂ absorption, which could lead to a rapid depletion of the liver's vitamin B₁₂ storage [28].

**Conclusions**

Based on our current studies, we conclude that vitamin B₁₂ is unrelated to gender, age, weight, or height. The HbA₁c test provides accurate results and can be a useful tool in determining diabetes diagnosis, although it is unrelated to the level of serum vitamin B₁₂. Vitamin B₁₂ deficiency is most common in patients with T₂DM who have been using metformin for a longer period of time and who are taking higher doses of metformin are taking higher doses of metformin.

**Recommendations**

Our data suggest the need for routine vitamin B₁₂ monitoring in patients with type-2 diabetes, especially in metformin users with an average dose of over 1,000 mg per day.

**Source of funding:** The current study was funded by our charges with no other funding sources elsewhere.

**Ethical clearance:** Ethical approval for this study was issued by the ethical committee of the Kurdistan higher counsel of medical specialist.

**Conflict of interest:** Nil

**References**


تحديد انتشار نقص فيتامين B12 في مرضى السكري (النوع الثاني) اللذين يعالجون بالعقار متيفورمين

ته لارم محمد، رقيب حسام الدين 1، 2، د. نوشيروان صادق محمد 3

الملخص

خلفية الدراسة: يعتبر مرض السكري من النوع الثاني مشكلة صحية عامة شائعة في عملية التمثيل الغذائي. يزيد العلاج المستمر بالمتيفورمين من مخاطر نقص فيتامين B12 وعواقبه الطبية على مرضى السكري من النوع الثاني.

أهداف الدراسة: للكشف عن انتشار نقص فيتامين B12 في الدم في مرضى السكري من النوع الثاني الذين تم علاجهم بالميتورمين أو بدونه.

المرضى والطريقة: أشتملت الدراسة على 200 حالة (100 مريض و 100 مجموعة ضابطة) استوفت المعايير الأساسية للدراسة. تم إجراء استبيان كامل وقياس مستوى فيتامين B12 في الدم. يُعرَف نقص فيتامين ب12 بأنه < 120 جزء من الغرام/مل في مصل الدم.

النتائج: تم الصرف على نقص فيتامين B12 في مصل الدم في 48٪ من المرضى (العدد = 48) ، بينما لم يكن مستويات HbA1c أي تأثير على مستوى فيتامين B12 في المصل للمرضى المعالجين بالمتيفورمين بجرعة 1 غم/يوم ، أظهر فرقًا كبيرًا مع هؤلاء المرضى الذين ليس لديهم تاريخ من استخدام الميتورمين.

الاستنتاجات: يرتبط المستوى المنخفض من فيتامين B12 في مصل الدم بأستخدام دواء الميتورمين لفترات اطول وجرعة اعلى.

الكلمات المفتاحية: مرض السكري، نمط -2 ميتورمين عوز فيتامين B12 في المصل.

البريد الإلكتروني: talarnaqshbandi@yahoo.com
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