# Vitamin B12 Deficiency Among a Sample of Type 2 Diabetic Patients on Metformin in Erbil-City

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## Abstract

**Background and objectives:** Metformin is the most common oral hypoglycemic agent used as first line together with life style modification in almost all type 2 diabetes patients worldwide. The aim of the study was to determine the percentage of vitamin B12 deficiency in a sample of patients with type 2 diabetes mellitus and its association with the daily dose metformin ($\geq$ 1.000 mg) and more than two years durations of treatment and anemia. **Methods:** A cross-sectional study was carried out on a sample of 100 adult patients with type 2 diabetes mellitus who attended the diabetic center and two General Teaching Hospitals in Erbil city during the period from the 1st of June to the end of December 2017. Vitamin B12 level, hemoglobin concentration, and other biochemical indicators of all recruited patients were obtained. **Results:** The mean age (+SD) of diabetic patients was 54.35 ($\pm$ 9.71) years and the range was between 24 to 75 years. More than half of the patients (57%) had poor glycemic control and about one third (29%) of patients had vitamin B12 level less than 200 pg/ml. Patients with obesity, longer duration of disease and on high dose of metformin had a significantly higher level of vitamin B12 deficiency. **Conclusions:** vitamin B12 deficiency is common among patients with type 2 diabetes mellitus especially among those using high dose of metformin for a long time. Therefore, identifying low vitamin B12 level among diabetic patients raises the concern to the patients for the future complications and challenge for the clinicians for management.

**Keywords:** Type 2 diabetes mellitus; Vitamin B12; Metformin; Anemia .

## Introduction

Diabetes mellitus has been documented as the most common endocrine disorder and metformin is the most frequently oral hypoglycemic agent prescribed for the patients with type 2 diabetes mellitus (T2DM)[1]. The current clinical guidelines, including the European Association for the Study of Diabetes (EASD) and the American Diabetes Association (ADA), accented on metformin as the first-line treatment choice together with lifestyle modifications and management of hypertension[2-4].

Apart from glycemic control, the effects of metformin on lipid metabolism, inflammation, and oxidative stress are well known[5-7]. In addition, it is helpful for weight loss and reduction of risk of myocardial infarction and morbidities of overweight in type 2 diabetic patients[8]. It has been documented that metformin suppresses production of hepatic glucose and improves insulin signaling in muscle, adipose tissue and hepatic[9,10].

Gastrointestinal disturbances like diarrhea and vomiting are the main side effects of metformin[11]. Those persons have renal, hepatic insufficiency and/or congestive heart failure and take metformin are at a greater risk of lactic acidosis[12]. These kinds of side effects commence shortly following metformin taking and disappear after drug cessation.

Several studies have established a link between long-term use of metformin with biochemical vitamin B12 deficiency and anemia[13]. Vitamin B12 has a key role in the formation of erythrocytes, physiology of nerve cells, and in the metabolism of homocysteine[14]. Vitamin B12 deficiency cause megaloblastic anemia and peripheral neuropathy also it is a risk factor for cardiovascular disease[15].

A number of studies have accented the metformin impact on reduction of mean levels of B12 serum[16-18]. The studies reported an association between the metformin dose and duration with vitamin B12 deficiency, although the exact mechanism of this pathway has not been identified yet. Possibly, it impedes the vitamin B12 absorption in the terminal ileum[14].

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The aim of the present study was to determine the percentage of B12 deficiency among a sample of T2DM patients.

**Patients and Methods**

A cross sectional study conducted among a sample of diabetic patients attending outpatients of Layla Qasim diabetic center, Hawler teaching hospital, and Rizgary teaching hospital for any consultation and were screened for vitamin B12 and other hematological parameters. A total of 100 patients were enrolled in the study after providing verbal informed consent.

The patients met eligibility criteria if they were 18 years and older; diagnosed with type 2 diabetes mellitus and on 1.0 gm of metformin for two or more years' duration. Pregnant women; alcoholics; known cases of megaloblastic anemia\ perniciuos anemia, patients with hypothyroidism, malabsorption, gastric or intestinal surgery, inflammatory bowel disease (IBD) and on vitamin B12 supplementation were excluded from the study. The collection of data was conducted over six months from June 2017 to December 2017.

The baseline information of the patients was obtained through the direct interview with the patients and reviewing the medical records, including socio-demographic characteristics, and some disease-related information. The biochemical indicators were measured through the biochemical tests in the medical lab of each hospital. The weight in (Kg) was measured by a scale with 0.02 sensitivity without shoes with light dress and height in centimeter in a standing position.

The normal range of 80-96 fL/red cells in adult for Mean Corpuscular Volume (MCV) was considered in this study. The reference range of hemoglobin concentration in adults was determined for men between 14.0 and 17.5 (mean 15.7) g/dL and for women between 12.3 and 15.3 (mean 13.8) g/dL20. The optimal level of HbA1c was determined when it equals to or less than 7%. The body mass index (BMI) was categorized as normal weight when it was between 18.5 and 24.9, overweight between 25.0 and 29.9, and obese equals to and greater than 30. The vitamin B12 deficiency was determined if the level is less than 200 pg/dL21.

Mean and standard deviation were calculated to summarize the parametric variables, median and interquartile range for nonparametric variables and the frequencies and percentages for categorical variables. The prevalence of vitamin B12 deficiency was examined in frequency and percentage. The association of BMI, MCV, HbA1c, and Hb with vitamin B12 deficiency levels was examined through the Chi-square and Fishers' exact test. The significant level was established when p-value was less than 0.05. The statistical analysis was done by SPSS version 24. Independent t-test were performed for statistical analyses. The numbers are mean ± S.D. for metformin and Sulfonylureas duration and median ± interquartile range for metformin dose.

The ethical approval of the present study was taken from Ethics Committee in Kurdistan Board for Medical Specialties. Participation in this study was voluntary and patients' responses and confidentiality of their personal information were protected through the study. Written consent form was taken from all patients before study commencement.

**Results**

The mean age of the patients was 54.35±9.71 years (range: 24-75). More than half of the patients were females (58.0%) and the majority were married (98.0%) and live in urban areas (86.9%). Similarly, the majority of the patients were non-smokers (69.0%) and had no chronic diseases (50.0%), a quarter of them were hypertensive, Table 1.

**Table (1):** Socio-demograpgic distribution of the patients with their chronic diseases.

| Age (year), Range: 24-75 | Mean: 54.35 | S.D: 9.71 |
|---|---|---|
|  | No. | % |
| Gender |  |  |
| Male | 42 | 42.0 |
| Female | 58 | 58.0 |
| Marital Statius |  |  |
| Single | 2 | 2.00 |
| Married | 98 | 98.0 |
| Residence |  |  |
| Urban | 86 | 86.0 |
| Rural | 14 | 14.0 |
| Smoking |  |  |
| Never smoker | 69 | 69.0 |
| Ex-smoker | 19 | 19.0 |
| Current smoker | 12 | 12.0 |
| Chronic disease |  |  |
| Hypertension | 27 | 27.0 |
| Ischemic Heart disease | 1 | 1.0 |
| Multi-disease | 22 | 22.0 |
| No chronic disease | 50 | 50.0 |
| Total | 100 | 100 |

The patients participated in the present study were on metformin and had type 2 diabetes mellitus since the last 7.64 years and were on metformin for the last 5.15 years with a median dose of 1700 grams. A small percentage of the patients were on insulin (9.0%) for the past 7.05 years. In addition, less than one-third of them were on Sulfonylureas (29.0%) over 9.41 years, Table 2.

**Table (2):** Distribution of the patients in relation to treatment and duration of the disease.

| Patients' Characteristics (n=100) | Mean ± S.D. |
|---|---|
| Disease duration (year) | 7.64±4.40 |
| Metformin duration use (year) | 5.15±2.11 |
| Metformin dose (gram) | 1781±606.93 |
| Insulin duration (year) | 7.05±2.90 |
| Sulfonylureas Duration (year) | 9.41±4.67 |
|  | No. (%) |
| Insulin use | 9 (9.0) |
| Sulfonylureas | 29 (29.0) |

The biochemical and anthropometric measurements were depicted in Table 3. The study showed that majority of the patients were overweight (62.0%), followed by a small percentage of obesity (11.0%). The percentage of vitamin B12 deficiency was 29%. More than half of the patients (57%) had HbA1C $\geq$ 7%. The abnormality in MCV was not high (38%), 21% macrocytic. Majority of male patients had an abnormality in hemoglobin concentration (80%) in contrast with 28% in females, Table 3.

**Table (3):** Biochemical and anthropometric measurements of disease-related in type 2 diabetic patients.

| Patients' Characteristics (n=100) | No. | % |
|---|---|---|
| BMI categories |  |  |
| Normal weight | 27 | 27.0 |
| Overweight | 62 | 62.0 |
| Obese | 11 | 11.0 |
| Vitamin B12 categories |  |  |
| Deficiency | 29 | 29 |
| Normal status | 71 | 71 |
| HbA1c |  |  |
| Optimal | 43 | 43 |
| Uncontrolled | 57 | 57 |
| MCV Categories |  |  |
| Normal MCV | 62 | 62 |
| Abnormal | 38 | 38 |
| Hb Categories |  |  |
| Males |  |  |
| Normal Hb | 20 | 20 |
| Abnormal | 80 | 80 |
| Females |  |  |
| Normal Hb | 72 | 72 |
| Abnormal | 28 | 28 |

The study showed that the patients with obesity were more prone have a vitamin B12 deficiency (p-value=0.026). Vitamin B12 of the patients with controlled blood glucose were more likely to be normal to a normal level (p-value=0.001). Similarly, the study showed that the female patients with normal Hb levels were more prone to have a normal level of vitamin B12 (p-value =0.025). The study did not show significant association between Vitamin B12 deficiency with MCV (p-value =0.359) and the male Hb levels (p-value =0.096). The study did not show that insulin, sulfonylureas use, and being smoker in combination with metformin are associated with a higher level of vitamin B12 deficiency, Table 4.

**Table (4):** Vitamin B12 levels in association with biochemical and anthropometric measures of the patient.

| Biochemical and anthropometric measures | Vitamin B12 No. (%) | | p-value (two-sided)* |
|---|---|---|---|
| | Deficient | Normal | |
| BMI Categories | | | 0.026 |
| Normal Weight | 6 (22.2) | 21 (77.8) | |
| Overweight | 16 (25.8) | 46 (74.2) | |
| Obese | 7 (63.6) | 4 (36.4) | |
| HbA1c Categories | | | 0.001 |
| Controlled | 5 (11.6) | 38 (88.4) | |
| Uncontrollable | 24 (42.1) | 33 (57.9) | |
| MCV Categories | | | 0.359 |
| Normal MCV | 20 (32.3) | 42 (67.7) | |
| Abnormal | 9 (23.7) | 29 (76.3) | |
| HB Categories | | | |
| Men | | | |
| Normal Hb | 1 (8.3) | 11 (91.7) | 0.096 |
| Abnormal | 10 (33.3) | 20 (66.7) | |
| Women | | | |
| Normal Hb | 7 (20.0) | 28 (80.0) | 0.025 |
| Abnormal | 11 (47.8) | 12 (52.2) | |
| Insulin Use | | | |
| Yes | 0 (0.0) | 9 (100) | 0.056 |
| No | 29 (31.9) | 62 (68.1) | |
| Sulfonylureas Use | | | 0.242 |
| Yes | 6 (20.7) | 23 (79.3) | |
| No | 23 (32.4) | 48 (67.6) | |
| Smoking | | | 0.540 |
| Never smoker | 22 (31.9) | 47 (68.1) | |
| Ex-smoker | 5 (26.3) | 14 (73.7) | |
| Current-smoker | 2 (16.7) | 10 (83.3) | |
| *Fishers' exact test was performed for statistical analysis. | | | |

The study showed that the patients with longer duration and a higher dose of metformin had more likelihood to have vitamin B12 deficiency (p-value0.001), however, no significant difference was found between those patients with different sulfonylureas duration (p-value=0.167). The study showed a strong negative correlation between vitamin B12 deficiency and metformin dose and duration, Table 5.

**Table (5):** Comparison of vitamin B12 levels in medication duration and dose.

| Biochemical and anthropometric measures | Vitamin B12 Deficiency Mean (± SD) | | p-value (two-sided) |
|---|---|---|---|
| | Deficient | Normal | |
| Metformin duration | 6.64±1.87 | 4.55±1.91 | <0.001 |
| Metformin dose | 2477.59±270.77 | 1496.48±456.64 | <0.001 |
| Sulfonylureas Duration | 11.17±2.71 | 8.96±5.00 | 0.167 |

## Discussion

The authors' aim in conducting the current study was to fill the evident gap of the prevalence of vitamin B12 in T2DM and its association with metformin dose and duration in Erbil city. The overall vitamin B12 deficiency was 29% in the current study. The study showed that obese patients were more likely to have vitamin B12 deficiency, those with optimal HbA1c was more likely to have a normal vitamin B12. In addition, the female patients with normal Hb were more likely to have a normal vitamin B12 level. Vitamin B12 deficiency has been investigated by other researchers across the world. For example, a study reported 29.66% of vitamin B12 deficiency in type 2 diabetic patients on metformin measured through biochemical laboratory tests[22] and another investigation reported 19%[23]. The authors did not find any study on vitamin B12 deficiency in type 2 diabetic patients in Iraq.

Regardless of the link establishment between metformin and vitamin B12 deficiency, the exact reason for this pathway has not been identified in the literature. The studies indicated that prevalence of vitamin B12 deficiency is between 5.8% and 52% worldwide[23-27].

The present study found that longer use of metformin is associated with a greater level of vitamin B12 deficiency which is in agreement with the literature[22, 23, 26, 27]. However, this must be kept in mind that extended use of metformin in combination with vitamin B12 could result in a challenge of peripheral neuropathy in patients with non-insulin-dependent diabetes. Neuropathy is a complication making health abnormality among 30% of patients with vitamin B12 deficiency and aged 40 years and older. It could lead to diminished sensory feeling of the feet of patients[28]. The evidence in the medical literature mentions that the long-term use of metformin affects on calcium-dependent vitamin B12 absorption[7]. It has been documented that vitamin B12 is conversely associated with dose and duration of metformin in type 2 diabetic patients[24].

The literature supports concurrent intake of metformin and proton pump inhibitors or H2-blockers as the links with vitamin B12 deficiency. The reason backs this concept that reduction in gastric acidity has a key role in vitamin B12 malabsorption. The reduction in acidity occurs through acid discharge by the parietal cells in patients taking these two medications. The reduction in gastric acid created by parietal cells has a key role in vitamin B12 breakdown[24, 29, 30].

Other factors could be associated with vitamin B12 deficiency in type 2 diabetic patients. For instance, a study reported that the smoker type 2 diabetic patients are more likely to have vitamin B12 deficiency compared to those non-smoker diabetic patients; 62.90% versus [27].989%; (p-value<0.001). We could not find this association in this study as the numbers of smokers in our sample were 2

persons only, which does not allow the investigators to make this kind of relationship[22].

The studies recommended a daily intake of vitamin 12 supplementation to prevent the possible vitamin B12 deficiency in patients on metformin as they are at greater risk of vitamin B12 deficiency. It was documented that the T2DM patients take multivitamin supplements were less likely to have vitamin B12 compared to nonusers (p-value<0.05)[22]. Daily use of vitamin B12 has a seminal role in the prevention of vitamin B12 deficiency. The literature confirmed that the vitamin B12 is raised following multivitamin supplementations. Between six to nine micrograms of vitamin B12 is recommended on regular basis to increase the vitamin level in the blood[31]. The current study showed that those female patients with normal hemoglobin concentration had a more likely to have a normal level of vitamin B12, while this association was not found among male patients. In agreement with the present study, another study found no significant difference in anemia between the patients with and without vitamin B12 deficiency (p-value=0.378)[27].

The findings reported in the present study must be interpreted in the inherence of study design and sampling methods as the cross-sectional study preclude us to make a causal pathway between vitamin B12 levels and type 2 diabetes. In addition, it maybe that the sample size of the study did not allow us to make other possible justifications. The findings may not be generalized to other settings in the rest of the country as the sample has been taken in one city only.

## Conclusions

The Percentage of vitamin B12 deficiency among a sample of T2DM was 29% in this study raises the concern to the patients for the possible future complications and challenge to the clinicians for management. In addition, the findings of the study suggest that the patients with longer duration of metformin use are at risk of vitamin B12 deficiency. Therefore, the doctors need to keep in mind to establish the screening strategies for these kinds of patients; in particular, those patients who visit the clinic with distressing neurologic symptoms.

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