**Araid Journal of Science and Technology (AJST )**
**Higher Institute of Science and Technology-Al Shmokh**

**مجلة الرائد للعلوم والتقنية**
المعهد العالي للعلوم والتقنية - الشموخ

*Received: 19 May 2026 | Accepted: 14 June 2026 | Published online: 30 June 2026*

# High Prevalence of Metformin-Associated Vitamin B12 Deficiency Among Type 2 Diabetic Patients in Libya: A Cross-Sectional Study

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## ABSTRACT

Metformin is a drug regularly used to treat type 2 diabetic patients by controlling blood glucose concentration. It is heavily linked to vitamin B12 deficiency. While this association has been thoroughly studied in Western demographics, Libyan data are lacking. Genetic, dietary, and environmental factors may result in different results than those found in other cohorts.

**KEYWORDS**: Vitamin B12; Metformin; Type 2 Diabetes.

## 1. INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a metabolic disorder caused by the body's inability to properly use insulin, resulting in hyperglycemia, and in turn increasing patients' risk of renal, neuropathic, and cardiovascular complications (American Diabetes Association, 2024).

Metformin (dimethylbiguanide), an oral medication used to reduce blood glucose concentrations in patients with T2DM, improves insulin sensitivity and thus reduces insulin resistance prevalent in type 2 diabetic patients. The drug has been known to interfere with vitamin B12 absorption through calcium-dependent ileal receptor antagonism (Ahmed & Obeid, 2023). Reportedly, the deficiency (serum B12 < 200 pg/mL) occurs in 10-30% of long-term users (Smith et al., 2023).

Nonetheless, while vitamin B12 deficiency induced by the use of metformin is well-documented in Asian, European, and North American populations (Almatraf et al., 2022), data from the North African region, specifically Libya, is scarce; no studies have assessed this association in Libya, where dietary and genetic profiles differ from previously studied demographics. From a dietary standpoint, Libyan diets are typically high in animal-based B12 sources (e.g. lamb and dairy) (Libyan Ministry of Health, 2021), which may counteract the prevalence of the deficiency.

Paradoxically, a lack of fortified foods has resulted in 41% of Libyans having low dietary vitamin B12 intake. Furthermore, TCN2 gene polymorphism (Zgheib et al., 2022) and H. pylori endemicity (Alzawi et al., 2023) may amplify risk of the deficiency among the population. This study therefore addresses a critical gap in Libyan public

health and in global understanding of the correlation between the deficiency and metformin, by providing the first Libyan-specific data set.

## Methods

### Study Design and Setting

This cross-sectional, laboratory-confirmed study was conducted by the department of pharmacy at the University of Tripoli in Tripoli, Libya, between October 15, 2023, and May 29, 2025. The laboratory analyses in this study were held at the Al-Yaqeen Medical Laboratory in Bani Walid and the Al-Qalb Medical Analysis Laboratory in Zliten (independent, private laboratories).

### Participants

The study enrolled 37 adults with clinically-confirmed diabetes currently using metformin and an additional 37 adults without diabetes (not taking metformin) based on the following criteria:

*Inclusion Criteria*

- Libyan adults (18 years or older)
- Diagnosed with type 2 diabetes (for the experimental group)
- Currently using metformin (for the experimental group)

*Exclusion Criteria*

- Type 1 diabetes
- Pregnancy
- History of gastrectomy or colon resection
- Non-diabetic status (for experimental group)

### Data Collection

Questionnaire: Demographic data of the participants was collected using questionnaires. General information regarding patients' gender and age was obtained. Duration of metformin use, the daily dose, and the results of laboratory tests were further acquired.

Specimen Preparation: A 2 ml venous blood sample was taken and placed in a tube that did not contain an anticoagulant. The serum was separated from the rest of the blood components by centrifugation. A 150-micron quantity of serum was frozen for approximately one month until it was used in the vitamin B12 analysis.

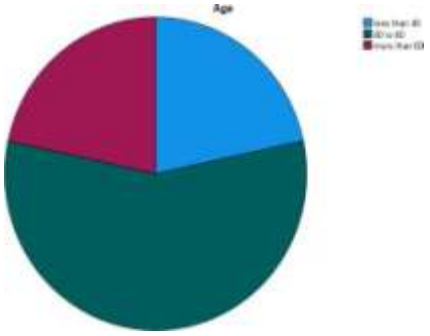
Laboratory Analysis: Serum B12 levels were measured using Clia-mate plus (Mindray CL-1200) chemiluminescence immunoassay reagent kits (normal range: 191-916 pg/mL; interassay CV
<5%).

## Results

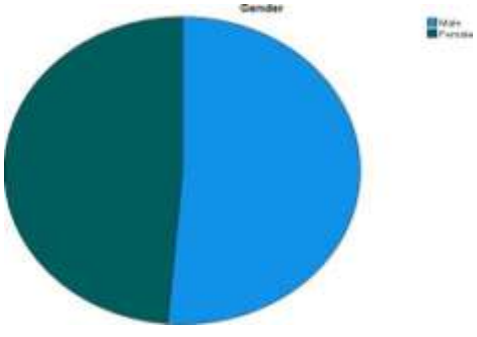
### Participant Demographic

This study included 37 T2DM patients on metformin (cases) and 37 non-diabetic controls. Of these participants 51.4% (n=19) were male and 48.6% (n=18) were female. There was nosubstantial difference in sex distribution between cases and controls (p=0.350). Furthermore, 56.8% (n=21) were between the ages of 40 and 60 years, 21.6% (n=8) were younger 40 years, and an additional 21.6% were older than 60 years. The control group contained a significantly younger demographic, which required statistical adjustment in later analyses.

| Age | Frequency | Percent |
|---|---|---|
| Younger than 40 years | 8 | 21.6 |
| 40-60 years | 21 | 56.8 |
| Older than 60 years | 8 | 21.6 |
| Total | 37 | 100.0 |



| Gender | Frequency | Percent |
|---|---|---|
| Male | 19 | 51.4 |
| Female | 18 | 48.6 |
| Total | 37 | 100.0 |



### Metformin Dosage

The most commonly used metformin doses were 1000 mg (32.4%) and 500 mg (35.1%). Combination doses (e.g. 50/500 mg) were less common but showed higher rates of deficiency.

| Dose | 500 mg | 850 mg | 50/500 mg | 50/850 mg | 50/1000 mg | 1000 mg | Total |
|---|---|---|---|---|---|---|---|
| Frequency | 13 | 7 | 1 | 1 | 3 | 12 | 37 |
| Percentage (%) | 35.1 | 18.9 | 2.7 | 2.7 | 8.1 | 32.4 | 100 |

**Vitamin B12 Levels and Deficiency**

Metformin users demonstrated substantially lower mean vitamin B12 levels (358.3 ± 158.9 pg/mL) than the controls (570.9 ± 190.2 pg/mL; $p<0.001$), with a mean difference of -212.5 pg/mL (95% Cl: -293.75, -131.29). 16.2% (n=6) of metformin users had vitamin B12 deficiency, while no controls were deficient. The results exhibited a notable relationship between dose and deficiency with 100% of patients taking 50/1000 mg (n=3) being deficient, and only 7.7% of those taking 500 mg (n=1/13) showed deficiency ($p<0.001$).

**The Relation Between the Experimental and Control Group**

| Gender | | Case | Control | P value | 95% Confidence Interval | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| | Male | 19(57.6%) | 14(42.4%) | 0.350 | 0.687 | 4.376 |
| | Female | 18(43.9%) | 23(56.1%) | | | |
| Total | | 37(50.0%) | 37(50.0%) | | | |

**The Relation of Vitamin B12 Value Between the Experimental and the Control Group**

| Group | | Mean | Std. Deviation | P value | 95% Confidence Interval of the Difference | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Case** | 7 | 358.3295 | 158.91282 | < 0.001 | -293.75 | -131.29 |
| **Control** | 7 | 570.8568 | 190.20654 | | | |

**The Relation Between Vitamin B12 and the Daily Metformin Dose**

| **Dose** | **500 mg** | **850 mg** | **50/500 mg** | **50/850 mg** | **50/1000mg** | **1000 mg** | **Total** |
|---|---|---|---|---|---|---|---|
| **Low** | 1 (7.7%) | 1(14.3%) | 0 | 1 (100%) | 3 (100%) | 0 | 6 (16.2%) |
| **Normal** | 12 (92.3%) | 6(85.7%) | 1(100.0%) | 0 | 0 | 12 (100.0%) | 31(83.8%) |
| **P-Value** | < 0.001 | | | | | | |

**Duration and Additional Medications**

Of the 37 cases in this study, 54.1% were taking metformin for less than 5 years prior to joining the study and 29.7% had used it for more than 10 years. Concomitant diabetes medications (e.g., Amaryl and Galvus) were taken by 29.7% of participants. Notably, 62.2% of the cases had former vitamin B12 testing, suggesting clinical suspicion of deficiency.

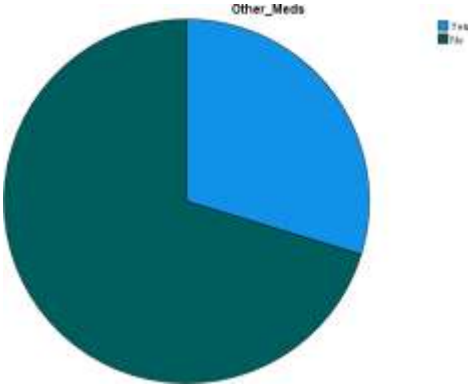
**Duration of Metformin Use**

| **Years of Use** | **Less than 5 Years** | **5-10 Years** | **More than 10 Years** | **Total** |
|---|---|---|---|---|
| **Frequency** | 20 | 6 | 11 | 37 |
| **Percentage (%)** | 54.1 | 16.2 | 29.7 | 100 |

**Other Medications**

| Other Medications? | Frequency | Percent |
|---|---|---|

| | | |
|---|---|---|
| Yes | 11 | 29.7 |
| No | 26 | 70.3 |
| Total | 37 | 100 |




**Taking Diabetes Medications**

| Diabetes Medication? If yes, what? | o | Amaryl 2 | Galvus 50 | Diamicron 60 | Sitagliptin 50 | Total |
|---|---|---|---|---|---|---|
| Frequency | 6 | 5 | 3 | 2 | 1 | 37 |
| Percent | 0.3 | 13.5 | 8.1 | 5.4 | 2.7 | 100 |

## Discussion

This study reveals that Libyan patients with T2DM using metformin have significantly lower vitamin B12 levels, with 16.2% having B12 levels lower than 200 pg/mL (358.3 pg/mL in cases as opposed to 570.9 pg/mL in controls).

## Key Interpretations

*Metformin's Dose-Dependent Effect*

Compared to other, more thoroughly studied regions in the middle east, Libyan data presents notable parallels and contrasts. The high deficiency rate (100%) among cases taking 50/1000 mg metformin doses, is mirrored in the Saudi Arabian cohort (Almatraf et al., 2022), but is contrasted by Indian data, where low deficiency rates (7.8%) are linked to widespread B12 supplementation (Raizada et al., 2017). This indicates that a lack of fortified foods plays a significant role in deficiency rates among the population.

*Genetic and Environmental Factors*

62% of participants reported prior B12 testing, which suggests clinical awareness. Yet, deficiency among the population persists suggesting that genetic and environmental factors may play a role in this epidemic. Genetic factors, such as TCN2 polymorphism, impair B12 transport (Zgheib et al., 2022) and might exacerbate the deficiency. Additionally, environmental cofactors, such as H. pylori infection (which have a 60% prevalence in the region) (Alzawi et al., 2023) may compound malabsorption.

*Age and Gender Neutrality*

Neither age nor gender predicted B12 levels post statistical adjustment (p=0.0350 for gender), which contrasts data in western cohorts (Green et al., 2022). This suggests that metformin's effect on B12 level overrides known risk factors in Libya.

**Clinical and Policy Recommendations**

The above discussed interpretations suggest a need for more proactive and effective vitamin B12 monitoring and supplementation methods on a national level. We advise that Libya' health ministry leverages its universal healthcare system to advocate routine screening for T2DM patients as per ADA 2024 guidelines. This is especially crucial for patients on metformin doses greater than 500 mg and patients who have been on the drug for longer than 5 years. High- dose users may require prophylactic B12, as observed in the United Kingdom's protocol (Langan et al., 2017).

## CONCLUSION

This cross sectional study conducted at the University of Tripoli examined the association between metformin (dimethylbiguanide) and vitamin B12 levels in type 2 diabetic Libyan adults and has provided the first evidence to link the use of metformin with the deficiency (serum B12<200 pg/mL) within the population, with 16.2% of type 2 diabetic users exhibiting the deficiency compared to none in the control group ($p<0.001$). Furthermore, the study uncovered a dose-dependent relationship, with 100% deficiency rate among cases taking high doses. These findings underscore a critical public health gap that must be addressed by a reassessment of protocol and monitoring of this deficiency among the type 2 diabetic Libyan community.

Moreover, it is imperative that larger studies are conducted to investigate environmental and genetic factors that exacerbate this deficiency. This study acts as a vital first step in understanding the link between T2DM and vitamin B12 deficiency among the North African population, an association that demands systemic mitigation.

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