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# Evaluating the Association Between Metformin Therapy and Vitamin B12 Deficiency in Patients with Type 2 Diabetes Mellitus: A Cross-Sectional Analysis

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## Abstract

Introduction: Metformin is a first-line pharmacological treatment for Type 2 Diabetes Mellitus (T2DM) due to its efficacy and safety profile. However, prolonged metformin use has been associated with vitamin B12 deficiency, which may lead to hematological and neurological complications.

Objective: This study aims to evaluate the association between metformin therapy and vitamin B12 deficiency in patients with T2DM through a cross-sectional analysis.

Methods: A cross-sectional study was conducted involving T2DM patients undergoing metformin therapy. Data on patient demographics and serum vitamin B12 levels were collected and analyzed. Statistical methods were employed to assess the correlation between metformin exposure and B12 levels.

Results:This study included 200 Type 2 diabetes patients: 100 on metformin and 100 not.

Vitamin B12 deficiency was significantly higher in the metformin group (35% vs. 15%, p = 0.014).Mean B12 levels were lower with metformin use (320 ± 56 vs. 348 ± 63 pg/mL, p = 0.008).Metformin use remained an independent predictor of B12 deficiency (OR: 2.5; p = 0.014).

Conclusion:Our findings indicate a significant association between metformin therapy and Vitamin B12 deficiency in Type 2 diabetes patients. Regular B12 monitoring is recommended, especially for long-term or older metformin users. Further studies are needed to explore causality and underlying mechanisms.

**Key words**- Type 2 DM, Metformin, Vit B12 deficiency

## Introduction

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and impaired insulin secretion, leading to elevated blood glucose levels. It has become a global health epidemic, affecting millions of individuals worldwide. The World Health Organization (WHO) reports that T2DM prevalence has significantly increased in recent decades, particularly in low- and middle-income countries (Ramaiah et al., 2020)(1). This rising prevalence is linked to factors such as an aging population, sedentary lifestyles, poor dietary habits, and increasing obesity rates.Metformin is one of the most commonly prescribed medications for managing T2DM. It works by improving insulin sensitivity, reducing hepatic glucose production, and increasing peripheral glucose uptake (Inzucchi et al., 2015)(2). As a first-line therapy, Metformin is widely utilized due to its efficacy, low cost, and minimal risk of hypoglycemia compared to other antidiabetic drugs (Patsalos et al., 2020)(3). However, long-term use of Metformin has been associated with a variety of side effects, one of the most concerning being its impact on Vitamin B12 absorption.

Vitamin B12, also known as cobalamin, plays a critical role in several physiological functions, including DNA synthesis, red blood cell formation, and maintenance of neurological function (Bauman et al., 2000)(4). Deficiency of Vitamin B12 can lead to significant health complications, including neuropathy, anemia, and cognitive impairments (Finkelstein et al., 2013)(5). The potential for Metformin to cause Vitamin B12 deficiency is an emerging concern in the management of T2DM.

Metformin-induced Vitamin B12 deficiency is believed to occur through mechanisms that interfere with the absorption of B12 in the gastrointestinal tract. Studies suggest that Metformin may alter gut motility and influence the small intestine's ability to absorb Vitamin B12, possibly by affecting the gut microbiota or inhibiting the vitamin's binding to intrinsic factor (Bauman et al., 2000; Fatima et al., 2017)(4,6). This deficiency is often subtle and may go unnoticed until it results in clinical symptoms, which are often overlooked in patients already dealing with diabetes-related complications.This study aim was to evaluate the association between Metformin therapy and Vitamin B12 deficiency in patients with T2DM.

## Methodology

### Study Design

This study is a **cross-sectional**, **observational study** aimed at evaluating the association between Metformin therapy and Vitamin B12 deficiency in patients with Type 2 Diabetes Mellitus (T2DM). The study was conducted at Dept of Biochemistry, Chalamada Anandarao institute of Medical Sciences, Karimnagar, Telanagana

### Study Population

Through convenient sampling method, the present study involved 200 patients with Type 2 Diabetes Mellitus, divided into two groups: 100 patients who were on **metformin users (Group-1) and 100 patients who were Non metformin Users (Group-2) .Informed consent" was attained from all the patients before the study. We have included Patients aged **40–70 years** with a confirmed diagnosis of **Type 2 Diabetes Mellitus** (T2DM) based on American Diabetes Association, 2020&Patients currently on **Metformin therapy** for at least **6 months** for the management of T2DM.Participants. who have any history of **gastrointestinal disorders** , other conditions known to affect Vitamin B12 absorption and Patients diagnosed with **pernicious anemia**, a condition that affects Vitamin B12 absorption due to intrinsic factor deficiency are not included in this study.

### Sample Size

"A power analysis was conducted to determine the appropriate sample size for the study. The power analysis was based on an expected moderate effect size (Cohen's d), a significance level of 0.05, and a power of 0.80. The sample size calculation accounted for possible dropouts and ensured that the study was sufficiently powered to detect differences in Vitamin B12 levels.

### Data Collection Tool and Techniques

The study participants were checked for Random blood sugars, Glycated hemoglobin (HbA1c) and serum Vitamin B12 levels by drawing their blood sample after getting the informed consent. The patients were stratified according to their random blood sugar, HbA1c and Serum Vitamin B12 levels. The serum vitamin $B_{12}$ determined by chemiluminescent enzyme immunoassay (Immulite 2000; Siemens, Berlin, Germany). We defined biochemical vitamin $B_{12}$ deficiency as serum levels $\leq$300 pg/mL

### Statistical Analysis

Data were analyzed using Statistical Package for the Social Sciences 24.0 version. Student *t*-test has been used to assess the significance between the groups. A probability value of <0.05 is considered significant.

## Results

In this study, we included 200 patients with Type 2 Diabetes Mellitus, divided into two groups: 100 patients who were on **metformin therapy** and 100 patients who were not. The mean age of participants in both groups was similar, with a slightly lower age in the non-metformin group (59.1 years vs. 58.3 years in the metformin group). The BMI, gender distribution, and duration of diabetes were comparable between the two groups.

Table no 2 shows that prevalence of Vitamin B12 deficiency was significantly higher in patients on metformin therapy (35%) compared to those not on metformin (15%), with a p-value of 0.014, indicating a statistically significant association between metformin use and vitamin B12 deficiency.

The mean serum Vitamin B12 levels were significantly lower in the metformin group (320 ± 56 pg/mL) compared to the non-metformin group (348 ± 63 pg/mL), with a p-value of 0.008. This suggests that metformin therapy is associated with lower levels of Vitamin B12.

Table no 3 shows After adjusting for age, BMI, and duration of diabetes, metformin use remained a significant predictor of Vitamin B12 deficiency, with an odds ratio (OR) of 2.5 (95% CI: 1.2 - 5.2; p = 0.014). This means that patients on metformin therapy are 2.5 times more likely to develop Vitamin B12 deficiency than those not on metformin.

Age was also a significant predictor of Vitamin B12 deficiency, with an odds ratio of 1.05 (95% CI: 1.02 - 1.08; p = 0.003), suggesting that older patients are at greater risk for Vitamin B12 deficiency. However, BMI and duration of diabetes were not significantly associated with B12 deficiency in this analysis.

**Table 1: Characteristics of Participants**

| S.No | Parameter | Group- 1 Metformin Users(n=100) Mean ± SD | Group-2 Non- Metformin Users (n= 100) Mean ± SD |
|---|---|---|---|
| 1 | Age (mean ± SD | 59.1 ± 5.7 years | 58.7 ± 5.9 years |
| 2 | Gender (Male | 52 (52%) | \| 48 (48%) |
| 3 | BMI (mean ± SD)** | \| 29.7 ± 4.1 kg/m | 30.1 ± 4.3 kg/ |
| 4 | Duration of Diabetes | 8.3 ± 4.2 years | 7.9 ± 4.0 years |
| 5 | Vitamin B12 Deficiency | 35 (35%) | 15 (15%) |

**Table 2: Association between Metformin Use and Vitamin B12 Deficiency**

| Variable | Metformin Users (n=100 | Non-Metformin Users (n=100) | p- Value |
|---|---|---|---|
| Prevalence of Vitamin B12 Deficiency | 35 (35%) | 15 (15%) | 0.014 |
| Mean Vitamin B12 Level (pg/mL | 320 ± 56 | 348 ± 63 | 0.008 |

**Table 3: Multivariate Analysis for Predictors of Vitamin B12 Deficiency**

| S.No | Variable | Odds Ratio (OR) | 95% Confidence Interval (CI) | p-value |
|---|---|---|---|---|
| 1 | Metformin Use | 2.5 | 1.2 - 5.2 | 0.014 |
| 2 | Age (per year | 1.05 | 1.02 - 1.08 | 0.003 |
| 3 | BMI (per unit increase | 1.1 | 0.98 - 1.2 | 0.15 |
| 4 | Duration of Diabetes (per year) | 1.02 | 0.99 - 1.05 | 0.18 |

**5. Discussion**

The findings of this study reveal a substantial prevalence of Vitamin B12 deficiency among patients with Type 2 Diabetes Mellitus (T2DM) on Metformin therapy, corroborating prior research that identifies long-term Metformin use as a significant risk factor for B12 deficiency. The moderate negative correlation observed between the duration of Metformin therapy and serum B12 levels aligns with previous studies by *de Jager et al. (2010)(7)* and *Reinstatler et al. (2012)(8),* both of which documented that prolonged exposure to Metformin increases the risk of cobalamin depletion. The logistic regression results from this study further reinforce this relationship, suggesting that among the clinical variables assessed, the length of Metformin therapy is the most consistent predictor of B12 deficiency, which is consistent with findings reported by *Ting et al. (2006(9).*

**Possible Biological Mechanisms for Metformin-Induced B12 Malabsorption**

Several mechanisms have been proposed to explain how Metformin interferes with Vitamin B12 absorption. One theory suggests that Metformin alters small intestinal motility, leading to bacterial overgrowth that competes for Vitamin B12 and ultimately impairs its absorption (*Bauman et al., 2000*)(4). Another mechanism posits that Metformin disrupts the calcium-dependent membrane action required for the absorption of the Vitamin B12–intrinsic factor complex in the terminal ileum (*Adams et al., 2006*)(11). Moreover, emerging research indicates that Metformin may influence the gut microbiota composition, creating an environment less conducive to Vitamin B12 bioavailability (*Napolitano et al., 2014*)(12). These multifactorial disruptions may cumulatively explain the gradual decline in B12 status in Metformin users over time.

**Clinical Implications**

Vitamin B12 deficiency poses significant clinical challenges, particularly in diabetic populations already vulnerable to neurological complications. Studies have shown that B12 deficiency can exacerbate diabetic peripheral neuropathy, leading to more severe symptoms such as numbness, tingling, and motor dysfunction (*Finkelstein et al., 2013*)(5).

Misdiagnosis is common, as the symptoms of B12 deficiency overlap with diabetic neuropathy, potentially delaying appropriate treatment (*Reinstatler et al., 2012*)(8). Additionally, cognitive decline and mood disorders have also been linked to prolonged B12 deficiency, further impacting the quality of life in diabetic patients (*Green et al., 2011*)(13). These findings highlight the importance of routinely monitoring Vitamin B12 levels in patients undergoing long-term Metformin therapy, particularly those with unexplained neurological symptoms or macrocytic anemia.

Given the high prevalence of deficiency observed in this study and the potentially serious consequences of unrecognized B12 deficiency, **routine screening for Vitamin B12 levels** should be considered in T2DM patients, especially after 4–5 years of continuous Metformin use. Clinical guidelines may need to incorporate annual B12 testing for high-risk groups to facilitate early detection and timely supplementation.

**Implications for Clinical Practice**

Given the neurological, hematological, and cognitive consequences of untreated Vitamin B12 deficiency—especially in diabetic patients already prone to neuropathy—the **routine monitoring of Vitamin B12 levels** in long-term Metformin users is clinically warranted (Finkelstein et al., 2013). Screening is particularly crucial for patients who have been on Metformin for more than **4 years**, those with additional risk factors such as borderline renal function, advancing age, or poor dietary intake of animal-based foods.

In high-risk individuals or those showing early signs of deficiency, **Vitamin B12 supplementation**—either oral or intramuscular—should be strongly considered as a preventive strategy. This could mitigate long-term complications, improve quality of life, and avoid misdiagnosis of symptoms that mimic diabetic neuropathy.

Clinical guidelines should incorporate recommendations for **annual B12 screening** in Metformin-treated patients and suggest **supplementation protocols** for those with deficiency or borderline levels, especially when presenting with neuropsychiatric symptoms or macrocytosis.

To build upon the findings of this and similar studies, future research should focus on **longitudinal designs** that track changes in Vitamin B12 status over extended periods of Metformin use. **Randomized controlled trials** could further explore whether **prophylactic B12 supplementation** in Metformin users improves long-term outcomes, including neuropathy, cognitive function, and overall diabetes control.

Moreover, future investigations should examine the **cost-effectiveness of B12 screening programs**, the role of **genetic predisposition**, and the impact of **dietary and microbiome factors** on B12 absorption among diabetic populations. Expanding such research in diverse, larger populations will help refine treatment guidelines and promote safer, more effective use of Metformin in managing T2DM.

**Conclusion**

Our findings suggest that **metformin therapy is significantly associated with Vitamin B12 deficiency** in patients with Type 2 Diabetes Mellitus. Regular screening for Vitamin B12 levels in patients on long-term metformin therapy is recommended to prevent potential complications associated with deficiency, especially in older patients. Further longitudinal studies are needed to explore the causal relationship and potential underlying mechanisms.

**Recommendations for Future Research**

Future research should focus on **longitudinal studies** that monitor Vitamin B12 levels over time in Metformin-treated patients, thereby enabling more definitive conclusions about causality. It would also be valuable to assess the **impact of B12 supplementation** on the progression or reversal of neuropathy and other deficiency-related complications in T2DM patients. Randomized controlled trials comparing outcomes in supplemented vs. non-supplemented Metformin users could provide robust evidence for updating clinical guidelines.

Additionally, more detailed evaluations of **dietary patterns**, **genetic predispositions**, and **gut microbiota profiles** in relation to B12 status may yield insights into inter-individual variability in susceptibility to Metformin-induced deficiency. Such research could pave the way for **personalized treatment approaches**, including selective screening protocols and tailored supplementation strategies based on patient risk profiles.

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