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Original research

# PROGNOSTIC VALUE OF METFORMIN TREATMENT CHARACTERISTICS FOR VITAMIN $B_{12}$ DEFICIENCY AMONG PATIENTS WITH TYPE 2 DIABETES MELLITUS

Nguyen Thanh Thuy[1], Le Van Chi[2], Lam Van Hoang[3]

[1]PhD Student, Hue University of Medicine and Pharmacy, Hue University, Vietnam

[2]Dept. of Internal Medicine, Hue University of Medicine and Pharmacy, Hue University, Vietnam

[3]Tam Anh Hospital, Ho Chi Minh City, Vietnam

**ABSTRACT**

***Background:*** *Long-term treatment of diabetes mellitus with metformin is associated with an increased risk of vitamin $B_{12}$ deficiency. This study aims to describe the prevalence of vitamin $B_{12}$ deficiency in patients with type 2 diabetes mellitus (T2DM) treated with metformin-containing regimens compared with newly diagnosed patients, and to determine the prognostic value of metformin treatment characteristics for vitamin $B_{12}$ deficiency.*

***Methods:*** *This cross-sectional study included 579 patients with T2DM aged 18 - 60 years: 283 patients receiving continuous metformin treatment for ≥ 6 months (patient group) and 296 newly diagnosed patients not yet treated with glucose-lowering medications (reference group). The study was conducted at the Outpatient Department of Cho Ray Hospital from January 2024 to August 2025. Receiver operating characteristic (ROC) analysis was used to evaluate the prognostic value for vitamin $B_{12}$ deficiency.*

***Results:*** *The prevalence of vitamin $B_{12}$ deficiency in the patient group was 14.1%, which was markedly higher than in the reference group (0.3%). The mean serum vitamin $B_{12}$ concentration in the patient group was 484.16 ± 202.80 pg/mL, lower than that in the reference group (666.94 ± 85.38 pg/mL). A metformin treatment duration exceeding 4.83 years and a metformin use index (MUI) greater than 8 showed high prognostic value for vitamin $B_{12}$ deficiency, with AUCs of 0.921 and 0.901, respectively, whereas metformin dose was not diagnostically significant.*

***Conclusion:*** *Patients with T2DM treated with metformin longer than 4.83 years and have metformin usage index (MUI) greater than 8 should undergo early screening for vitamin $B_{12}$ deficiency to reduce treatment-related complications and to improve the comprehensive management of T2DM.*

***Keywords:*** *Type 2 diabetes mellitus, metformin, vitamin $B_{12}$ deficiency, prognostic value.*

## I. INTRODUCTION

Diabetes mellitus is a group of metabolic disorders with a rapidly increasing global prevalence [1, 2]. Poorly controlled type 2 diabetes mellitus (T2DM) can lead to a wide range of acute complications, including diabetic ketoacidosis, coma, hyperglycemia, and hypoglycemia, as well as chronic complications such as macrovascular disease, diabetic retinopathy, nephropathy, neuropathy, and diabetic foot disease [3]. Consequently, achieving optimal glycemic control remains a central goal in diabetes management, as it reduces complications and improves patients' quality of life. Among currently available glucose-lowering agents, metformin is recommended as the first-line therapy and is widely prescribed for the majority of patients with T2DM [1, 4, 5].

Beyond its well-established efficacy and safety profile, increasing evidence over recent decades has demonstrated an association between long-

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**Corresponding author:** Le Van Chi. Email: lvchi@huemed-univ.edu.vn. Phone: +84 913426457

term metformin use and reduced serum vitamin $B_{12}$ levels, thereby increasing the risk of vitamin $B_{12}$ deficiency. Although the exact pathophysiological mechanisms underlying metformin-related vitamin $B_{12}$ deficiency have not been fully elucidated, available data suggest that this condition likely results from multiple interacting mechanisms, including alterations in vitamin $B_{12}$ absorption and metabolism induced by metformin.

Clinically, vitamin $B_{12}$ deficiency often develops insidiously but may lead to serious consequences, such as megaloblastic anemia and neurological manifestations, including peripheral and autonomic neuropathy, loss of deep tendon reflexes, impaired proprioception, and reduced vibration sensation. In patients with T2DM, vitamin $B_{12}$ deficiency may further exacerbate pre-existing diabetic neuropathy, potentially resulting in a mixed and progressive neuropathy related to both diabetes and metformin-induced vitamin $B_{12}$ deficiency [6].

To date, no specific consensus guidelines recommend routine screening for vitamin $B_{12}$ deficiency in patients receiving metformin therapy. Consequently, this condition is frequently underdiagnosed or detected at a late stage. Several studies have reported that the risk of vitamin $B_{12}$ deficiency is associated with metformin treatment characteristics, such as daily dose, duration of therapy, and cumulative exposure assessed by the metformin use index [6]. However, findings across studies remain inconsistent, and most investigations have focused primarily on associations rather than the prognostic value of these treatment-related factors for vitamin $B_{12}$ deficiency. Moreover, in Vietnam, studies systematically evaluating the prognostic role of metformin treatment characteristics in predicting vitamin $B_{12}$ deficiency among patients with T2DM are scarce.

In response to gaps in the existing literature, this study aimed to address both scientific and clinical needs by describing the prevalence of vitamin $B_{12}$ deficiency among patients with T2DM treated with metformin-containing regimens compared with newly diagnosed patients, and by evaluating the prognostic value of metformin treatment characteristics for vitamin $B_{12}$ deficiency. The findings are expected to inform the development of targeted monitoring and screening strategies tailored to clinical practice in Vietnam, thereby optimizing treatment outcomes and supporting personalized care for patients with T2DM.

## II. MATERIAL AND METHODS

### 2.1. Study Population

The study population comprised patients, aged 18 - 60 years, who were diagnosed with T2DM at the Outpatient Department of Cho Ray Hospital from January 2024 to August 2025.

Inclusion Criteria:

Patients with T2DM who agreed to participate were allocated to two groups as follows:

- Patient group: Patients previously diagnosed with T2DM who had been receiving continuous metformin-based therapy for at least six months and provided written informed consent.

- Reference group: Patients newly diagnosed with T2DM who had not previously received glucose-lowering agents, were age-matched to the patient group, and provided written informed consent.

Exclusion Criteria:

Participants were excluded if they met any of the following criteria: pregnancy; chronic alcohol abuse (daily alcohol consumption); adherence to a strict vegan diet; untreated or recently treated (within the previous three months) *Helicobacter pylori* infection; continuous use of proton pump inhibitors, $H_2$ receptor antagonists, or antacids for 12 months or longer; history of partial or total gastrectomy, bariatric surgery, pancreatectomy, ileal resection, or chronic enteropathies (e.g., Crohn's disease or terminal ileitis); liver cirrhosis; type 1 diabetes mellitus; hypothyroidism; heart failure or acute coronary syndrome within the previous three months; history of hemolytic anemia; severe acute infection or critical illness requiring hospitalization; current malignancy with ongoing treatment; active tuberculosis receiving para-aminosalicylic

acid therapy; use of drugs affecting metabolism (including cholestyramine, colchicine, neomycin, methotrexate, nicotinic acid, or oral contraceptives) within the previous three months; supplementation with vitamins $B_2$, $B_6$, $B_{12}$, and/or calcium within the past six months; refusal to participate; eosinophilia; aspartate aminotransferase (AST) or alanine aminotransferase (ALT) levels ≥ 3 times the upper limit of normal; estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m²; or folate deficiency, defined as a serum folate concentration < 5.3 ng/mL.

**2.2. Study design and sampling**

This cross-sectional study used convenience sampling. All patients who met the inclusion criteria and had no exclusion criteria during the study period were eligible for enrollment.

**2.3. Study variables**

The diagnosis of diabetes was based on the American Diabetes Association (ADA) 2024 criteria for non-pregnant adults, defined by one of the following: (a) Hemoglobin A1c (HbA1c) ≥ 6.5% using a standardized assay; (b) fasting plasma glucose ≥ 126 mg/dL (7.0 mmol/L) after at least 8 hours of fasting; (c) two-hour plasma glucose ≥ 200 mg/dL (11.1 mmol/L) during a 75-g oral glucose tolerance test; (d) or random plasma glucose ≥ 200 mg/dL (11.1 mmol/L) in the presence of classic symptoms of hyperglycemia. Patients previously diagnosed with T2DM and receiving treatment according to medical records were also classified as having diabetes. In the absence of classic symptoms, at least two abnormal test results from the same or separate samples were required to confirm the diagnosis [7].

Serum vitamin $B_{12}$ concentrations were measured using the reference range of 211 - 911 pg/mL established by the Biochemistry Department of Cho Ray Hospital. Vitamin $B_{12}$ deficiency was defined as a serum vitamin $B_{12}$ level < 211 pg/mL.

The metformin usage index (MUI) was calculated as the product of the daily metformin dose (mg) and the duration of use (years), divided by 1,000. The MUI was applied to patients receiving metformin therapy for at least six months, as proposed by Shivaprasad et al. (2020) [8].

**2.4. Data collection and analysis**

Data were collected, coded, entered, and analyzed using SPSS software version 20.0. Categorical variables were summarized as frequencies (n) and percentages (%), while continuous variables were expressed as means and standard deviations (SD). Receiver operating characteristic (ROC) analyses were performed to evaluate the prognostic value of metformin treatment characteristics for vitamin $B_{12}$ deficiency, including determination of optimal cut-off values using the Youden index, sensitivity, specificity, area under the curve (AUC), and 95% confidence intervals (CI) for AUC.

**2.5. Ethical Considerations**

The study was approved by the Institutional Ethics Committee of Hue University of Medicine and Pharmacy, Hue University (No. H2023/475, dated October 6, 2023). Participation was voluntary, and the study was designed and conducted to ensure that diagnosis and treatment were not delayed and that no additional costs were incurred by patients.

**III. RESULTS**

**3.1. General and treatment characteristics of the study participants**

A total of 579 patients meeting the inclusion criteria were enrolled at the Outpatient Department of Cho Ray Hospital during the study period. Of these, 283 patients had a prior diagnosis of T2DM and had been receiving continuous metformin-containing regimens for at least six months (patient group), while 296 patients were newly diagnosed with T2DM and had not yet initiated glucose-lowering therapy (reference group). The overall mean age of the study population was 50.32 ± 7.17 years, with individuals aged 50 years or older accounting for 59.4% of participants. The sex distribution was nearly equal, comprising 49.1% males and 50.9% females. No statistically significant differences were observed between the patient and reference groups with respect to mean age, age distribution, or sex composition ($p > 0.05$). Participant characteristics are summarized in Table 1.

**Table 1:** Characteristics of the study participants

| Characteristics | | Patient group (n = 283) | | Reference group (n = 296) | | Total (n = 579) | | p-value |
|---|---|---|---|---|---|---|---|---|
| | | n | % | n | % | n | % | |
| Age (Mean ± SD)<br>Min - Max | | 50.70 ± 7.10<br>22 - 60 | | 49.96 ± 7.20<br>23 - 60 | | 50.32 ± 7.17<br>22 - 60 | | > 0.05[a] |
| Age group | < 35 | 7 | 2.5 | 9 | 3.0 | 16 | 2.8 | > 0.05[b] |
| | 35 - < 50 | 101 | 35.7 | 118 | 39.9 | 219 | 37.8 | |
| | ≥ 50 | 175 | 61.8 | 169 | 57.1 | 344 | 59.4 | |
| Sex | Male | 143 | 50.5 | 141 | 47.6 | 284 | 49.1 | > 0.05[b] |
| | Female | 140 | 49.5 | 155 | 52.4 | 295 | 50.9 | |

[a]*Independent-samples t test,* [b]*Chi-squared test, SD: Standard deviation, Min: Minimum, Max: Maximum*

As shown in Table 2, the mean metformin dose was 1608.24 ± 441.34 mg per day, with doses of 1500 - 2000 mg per day accounting for the majority of prescriptions (72.1%). The mean duration of metformin treatment was 3.66 ± 3.15 years, and most patients had received metformin therapy for 6 months to less than 3 years (51.2%). The MUI was 6.18 ± 5.68, with over half of the patients having an MUI < 5 (55.5%).

**Table 2:** T2DM treatment characteristics with metformin among patient group (n = 283)

| T2DM treatment characteristics | | n | % |
|---|---|---|---|
| Current metformin dosage (mg per day) | 500 - < 1500 | 79 | 27.9 |
| | 1500 - 2000 | 204 | 72.1 |
| | (Mean ± SD)<br>Min - Max | 1608.24 ± 441.34<br>500 - 2000 | |
| Duration of metformin treatment | 6 months - < 3 years | 145 | 51.2 |
| | ≥ 3 years | 138 | 48.8 |
| | (Mean ± SD)<br>Min - Max | 3.66 ± 3.15<br>0.50 - 15.00 | |
| MUI | < 5 | 157 | 55.5 |
| | 5 - < 10 | 77 | 27.2 |
| | 10 - < 15 | 25 | 8.8 |
| | ≥ 15 | 24 | 8.5 |
| | (Mean ± SD)<br>Min - Max | 6.18 ± 5.68<br>0.25 - 28.50 | |

*SD: Standard deviation, Min: Minimum, Max: Maximum, MUI: Metformin usage index*

### 3.2. Serum vitamin $B_{12}$ deficiency in patients with T2DM

The mean serum vitamin $B_{12}$ concentration in the patient group was lower than that in the reference group (484.16 ± 202.80 pg/mL vs. 666.94 ± 85.38 pg/mL). The prevalence of vitamin $B_{12}$ deficiency was higher in the patient group compared with the reference group (14.1% vs. 0.3%). In addition, no

significant differences were observed in the prevalence of vitamin $B_{12}$ deficiency or mean serum vitamin $B_{12}$ concentration by sex between the two groups (Table 3).

**Table 3:** Stratification of serum vitamin $B_{12}$ concentrations by sex and study groups

| **Vitamin $B_{12}$ deficiency** | **Patient group (n = 283)** | | **Reference group (n = 296)** | | **p-value** |
|---|---|---|---|---|---|
| | **n** | **%** | **n** | **%** | |
| No | 243 | 85.9 | 295 | 99.7 | < 0.05[a] |
| Yes | 40 | 14.1 | 1 | 0.3 | |
| Mean ± SD (pg/mL) | 484.16 ± 202.80 | | 666.94 ± 185.38 | | < 0.05[b] |

| **Vitamin $B_{12}$ deficiency** | **Male (n = 143)** | | **Female (n = 140)** | | **Male (n = 141)** | | **Female (n = 155)** | |
|---|---|---|---|---|---|---|---|---|
| | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** |
| No | 120 | 83.9 | 123 | 87.9 | 141 | 100.0 | 154 | 99.4 |
| Yes | 23 | 16.1 | 17 | 12.1 | 0 | 0 | 1 | 0.6 |
| p-value | > 0.05[a] | | | | > 0.05[c] | | | |
| Mean ± SD (pg/mL) | 489.39 ± 203.85 | | 478.81 ± 202.31 | | 669.06 ± 175.06 | | 665.00 ± 194.85 | |
| p-value | > 0.05[b] | | | | > 0.05[b] | | | |

*SD: Standard deviation*

**3.3. The prognostic value of metformin treatment characteristics for vitamin $B_{12}$ deficiency**

As presented in Table 4 and Figure 1, the duration of metformin treatment and the MUI demonstrated good prognostic value for vitamin $B_{12}$ deficiency, with AUCs of 0.921 and 0.901, respectively. A treatment duration of > 4.83 years and an MUI of > 8 yielded high sensitivity and specificity for predicting vitamin $B_{12}$ deficiency (90.00% and 88.89%; 87.50% and 83.54%, respectively). In contrast, metformin dose showed no significant prognostic value for vitamin $B_{12}$ deficiency (AUC = 0.579).

**Table 4:** The prognostic value of metformin treatment characteristics for vitamin $B_{12}$ deficiency in the patient group (n = 283)

| **Metformin treatment characteristics** | **Cut-off point** | **Sensitivity** | **Specificity** | **AUC** | **95% CI** | **p-value** |
|---|---|---|---|---|---|---|
| Current metformin dosage (mg per day) | > 1583.33 | 75.00 | 39.92 | 0.579 | 0.519 - 0.637 | > 0.05 |
| Duration of metformin treatment (years) | > 4.83 | 90.00 | 88.89 | 0.921 | 0.883 - 0.950 | < 0.05 |
| MUI | > 8 | 87.50 | 83.54 | 0.901 | 0.860 - 0.933 | < 0.05 |

*AUC: Area under the curve, CI: confidence interval, MUI: Metformin usage index*

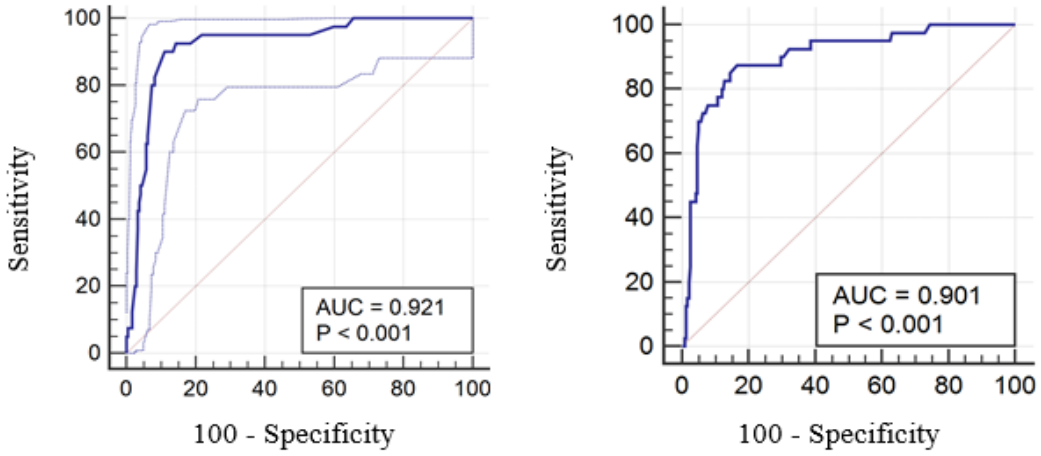


**Figure 1:** ROC curves showing the prognostic value of metformin treatment characteristics for vitamin $B_{12}$ deficiency in the patient group (n = 283)

## IV. DISCUSSION

### 4.1. Serum vitamin $B_{12}$ deficiency in patients with T2DM treated with metformin

In our study, the prevalence of serum vitamin $B_{12}$ deficiency in the patient group was 14.1%, which was significantly higher than that observed in the reference group at 0.3%. In addition, the mean serum vitamin $B_{12}$ concentration in the patient group was markedly lower than that of the reference group, with values of 484.16 ± 202.80 pg/mL and 666.94 ± 85.38 pg/mL, respectively. The prevalence of vitamin $B_{12}$ deficiency among patients with T2DM receiving metformin in our study was lower than that reported in several previous studies, including those by Jiwoon Kim (2019) [9], Fathy Elsayed Abdelgawad (2019) [10], Gayathri Devi Krishnan (2020) [11], Tahir Ullah Khan (2021) [12], Andrew Kien Han Wee (2023) [13], Dat Tan Huynh (2024) [14], and Hen Huu Phan (2025) [15], with reported prevalence rates ranging from 18.6% to 28.3%. These differences may be partly explained by variations in study design, population characteristics, duration of metformin use, and criteria used to define vitamin $B_{12}$ deficiency. However, our findings are comparable to those reported by Abu Kamran Rahul, Leili Gao, and Abonyi Michael Chinweuba [16, 17]. Abu Kamran Rahul (2023) reported a vitamin $B_{12}$ deficiency prevalence of 16% among metformin users, which was higher than that observed in non-users at 6%, although the difference did not reach statistical significance. In addition, the mean serum vitamin $B_{12}$ concentration was lower in metformin users than in non-users, with values of 481.52 pg/mL and 600.85 pg/mL, respectively [16]. Similarly, Abonyi Michael Chinweuba (2023) reported a vitamin $B_{12}$ deficiency rate of 16.6% using a cutoff value of less than 200 pg/mL [17].

The mechanisms underlying metformin-associated vitamin $B_{12}$ deficiency remain incompletely elucidated and are likely multifactorial. Specifically, metformin is thought to interfere with the binding of the intrinsic factor-vitamin $B_{12}$ complex to the cubilin receptor in the ileum through a calcium-dependent receptor pathway. Metformin may also interact directly with the cubilin receptor, thereby reducing vitamin $B_{12}$ absorption. In addition, metformin may alter small-intestinal motility, promoting bacterial overgrowth that impairs absorption of the intrinsic factor-vitamin $B_{12}$ complex in the terminal ileum. Other proposed mechanisms include disturbances in bile acid metabolism and reabsorption, increased hepatic sequestration of vitamin $B_{12}$, and reduced intrinsic factor secretion from gastric parietal cells [6].

There is currently no consensus on the diagnostic threshold for vitamin $B_{12}$ deficiency. Some authors define deficiency as a serum vitamin $B_{12}$ concentration below 150 pg/mL [12], whereas others apply higher thresholds, including less than 200 pg/mL [17] or

less than or equal to 300 pg/mL [11, 14, 15]. In the present study, vitamin $B_{12}$ deficiency was defined as a serum vitamin $B_{12}$ concentration below 211 pg/mL, based on the reference interval used by the Department of Biochemistry at Cho Ray Hospital, which is accredited and complies with ISO standard requirements.

**4.2. The prognostic value of metformin treatment characteristics for vitamin $B_{12}$ deficiency**

ROC analysis demonstrated that a metformin treatment duration exceeding 4.83 years had high prognostic value for vitamin $B_{12}$ deficiency, with an area under the curve of 0.921, high sensitivity, and high specificity, indicating excellent discriminative ability. Similarly, an MUI value greater than 8 also showed strong prognostic value, with an area under the curve of 0.901 and favorable sensitivity and specificity. Taken together, these findings highlight the important role of both treatment duration and cumulative metformin exposure in predicting the risk of vitamin $B_{12}$ deficiency. In line with the ADA recommendations, metformin remains widely prescribed as first-line therapy for T2DM because of its efficacy, safety, affordability, and accessibility. However, reduced vitamin $B_{12}$ absorption leading to deficiency is a well-recognized adverse effect of long-term metformin use [5]. In this context, Shivaprasad et al. proposed the MUI as a practical index for assessing the risk of vitamin $B_{12}$ deficiency in patients with T2DM treated with metformin for at least six months [8].

In contrast, metformin dose did not show prognostic value for vitamin $B_{12}$ deficiency in our study. This finding may be explained by the relatively homogeneous dosing pattern observed, as most participants received a moderate daily dose of metformin. These results further suggest that the effect of metformin on the development of vitamin $B_{12}$ deficiency is driven primarily by treatment duration rather than daily dose, thereby supporting a cumulative exposure mechanism. This interpretation is consistent with previous studies reporting a strong association between longer metformin use and vitamin $B_{12}$ deficiency [11, 16-18]. Gayathri Devi Krishnan reported that metformin use exceeding five years was associated with a significantly increased risk of vitamin $B_{12}$ deficiency [11]. Similarly, Abu Kamran Rahul identified a significant association between vitamin $B_{12}$ deficiency and the duration of metformin therapy [16], while Abonyi Michael Chinweuba reported that metformin use for more than five years was a major risk factor for vitamin $B_{12}$ deficiency [17]. In addition, Hurley-Kim demonstrated that each additional year of metformin use increased the risk of vitamin $B_{12}$ deficiency by 5%, and that a treatment duration of four years or longer markedly increased this risk to 41% compared with shorter exposure [18].

Nevertheless, our findings differ from those of several domestic and international studies that reported a significant role for both metformin dose and duration [14, 15, 19]. Dat Tan Huynh found that metformin dose and treatment duration were both significantly associated with vitamin $B_{12}$ deficiency [14]. Similarly, Hen Huu Phan reported that both factors were independently and strongly associated with vitamin $B_{12}$ deficiency [15]. Moreover, Leili Gao identified high daily metformin dose as an important determinant of metformin-induced vitamin $B_{12}$ deficiency [19]. This difference may be explained by the limited variability in daily metformin doses in our study population, as most patients received moderate and relatively uniform doses (500 - 2000 mg/day), which may have reduced the ability to detect a dose-response relationship.

Several limitations of the present study should be acknowledged. The cross-sectional design precludes causal inference, while the use of convenience sampling and a single-center setting may limit the generalizability of the findings to the broader Vietnamese population with T2DM. Future studies employing longitudinal designs are needed to confirm the causal relationship between long-term metformin use and vitamin $B_{12}$ deficiency. Newly diagnosed T2DM patients in the reference group may differ from patients treated for at least six months in certain respects. In addition, the absence of nationally standardized reference intervals for serum vitamin $B_{12}$ testing necessitated reliance on the reference range used by the Department of Biochemistry at Cho Ray Hospital, which may further limit the representativeness of our results.

## V. CONCLUSION

In conclusion, vitamin $B_{12}$ deficiency was observed in 14.1% of patients treated with metformin for at least six months, while it was rare among those without prior use of glucose-lowering agents, accounting for 0.3%. Longer metformin treatment duration exceeding 4.83 years and an MUI value above 8 showed strong prognostic value for vitamin $B_{12}$ deficiency, whereas metformin dose was not predictive. These findings highlight the importance of early and regular screening for vitamin $B_{12}$ deficiency in patients with T2DM treated with metformin at Cho Ray Hospital, which may help reduce related complications and improve diabetes management.

## Conflict of interest

The authors declare that they have no conflicts of interest related to the content of this study.

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