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ASSOCIATION BETWEEN VITAMIN B12 DEFICIENCY AND METFORMIN USE: A CROSS-SECTIONAL STUDY IN A TERTIARY HOSPITAL

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ASSOCIATION BETWEEN VITAMIN B12 DEFICIENCY AND METFORMIN USE: A CROSS-SECTIONAL STUDY IN A TERTIARY HOSPITAL

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ABSTRACT

Background: Vitamin B12 deficiency is a common and neglected adverse effect experienced by type 2 diabetic patients treated with metformin. A lack of vitamin B12 may have severe consequences, such as fatigue, macrocytic anemia, and worsened peripheral diabetic neuropathy. This study aims to identify the rate of vitamin B12 deficiency and the associated factors in type 2 diabetic patients treated with metformin.

Materials and methods: This cross-sectional study was conducted with 145 type 2 diabetic patients treated with metformin. Levels of vitamin B12 and associated clinical and paraclinical parameters were measured and collected.

Results: The study population's mean age was 61.5, of which 51.7% were female. The median duration of metformin use was 10 years, and the median metformin dose was 1700 mg. The vitamin B12 deficiency rate was 22.1%. HbA1c, duration of metformin use, and metformin dose were independent factors statistically associated with vitamin B12 deficiency.

Conclusion: This study identified the rate of vitamin B12 deficiency and associated factors in type 2 diabetic patients treated with metformin. These findings can be helpful in screening patients and replacing vitamin B12 in high risk populations with vitamin B12 deficiency.

Keywords: metformin; vitamin B12 deficiency; type 2 diabetes mellitus; Vietnam

INTRODUCTION

Metformin belongs to the biguanide class and is synthesized from galegine, an isoprenyl derivative of guanidine found in the perennial herb French lilac (*Galega officinalis*)[1]. Metformin is recommended in most treatment guidelines as the first-line drug for the management of type 2 diabetes, based on its effectiveness in blood glucose control, low cost, safety, and cardiovascular benefits, as demonstrated by the UK Prospective Diabetes Study[2,3].

The drug's effectiveness is dose-dependent, and the mechanism of action of metformin is not fully understood. Extensive evidence obtained in clinical studies and animal models suggests that the main function of metformin in regulating glucose homeostasis is to inhibit hepatic glucose production through the regulation of hepatic gluconeogenesis and glycogenolysis[4,5]. Metformin also improves cellular sensitivity to insulin, thereby enhancing peripheral glucose uptake, mainly in skeletal muscle, and significantly reducing fasting plasma insulin levels[6]. Improvements in insulin sensitivity with metformin may be due to its effects on insulin receptor expression and tyrosine kinase activity.

The most common adverse effects of metformin are gastrointestinal symptoms, such as diarrhea, nausea, and/or abdominal discomfort[7]. These symptoms are usually mild, transient, and dose-dependent. A rare but more concerning adverse effect of biguanides is lactic acidosis. Risk factors for metformin-induced lactic acidosis include conditions that increase lactate production, such as severe infections, severe liver or kidney disease, recent surgery, and any condition causing hypoxia or decreased tissue perfusion[8,9].

In 1969, Berchtold et al. first reported vitamin B12 deficiency in patients using metformin, resulting from decreased gastrointestinal vitamin B12 absorption[10]. This effect can occur as early as the third month of using metformin. Several mechanisms have been proposed to explain the effect of metformin, including changes in small intestinal motility leading to overgrowth of intestinal bacteria, competitive inhibition and inactivation of vitamin B12 absorption capacity, alteration of intrinsic factor levels, and interaction with the

intracellular cubilin receptor[11,12]. Of these, the most widely accepted mechanism is the inhibition of the calcium-dependent binding of the B12-intrinsic factor complex to the cubilin receptor in the ileum. The HOME study is one of the first clinical trials that confirmed the causative effect of vitamin B12 deficiency by metformin. The results showed that metformin usage at a dose of 850 mg three times daily reduces vitamin B12 levels by 19% compared to a placebo[13].

Type 2 diabetes mellitus is a complicated, heterogeneous, and multifactorial disease of which treatment often requires the combination of metformin and other medications[14–19], however, there are few studies on vitamin B12 deficiency related to metformin use in Vietnam. In 2023, Huynh et al. conducted a cross-sectional study on the prevalence of vitamin B12 deficiency in type 2 diabetic patients using metformin at the Vinmec Central Park Hospital, a private outpatient clinic in Ho Chi Minh City[20]. Of the 156 type 2 diabetic patients treated with metformin, the rate of vitamin B12 deficiency was 18.6%. Vitamin B12 deficiency may differ depending on region, diet, and studied population; this study set out to investigate the rate of vitamin B12 deficiency and its associated factors in type 2 diabetic patients treated with metformin in Cho Ray Hospital, one of the largest tertiary hospitals in Vietnam.

MATERIALS AND METHODS

1. Participant recruitment

The protocol for this cross-sectional study was approved by the Ethical Committee of Cho Ray Hospital (approval number 1711/CN-HĐĐĐ). A sample size calculation was performed to estimate the rate of vitamin B12 deficiency in the target population using the formula below:

$$N = Z^2_{(1-\frac{\alpha}{2})} \frac{p(1-p)}{d^2}$$

In which, $Z = 1.96$, $p = 0.095$ (based on a similar study by Ko et al.[21]), and $d = 0.05$. The estimated patient number was 132. Recruitment took place from March to August 2024. The main inclusion criteria were type 2 diabetic patients treated with metformin for at least 3 months with unchanged dose in the last 3 months. Patients were excluded from the study if they had (i) a recent acute illness, such as severe infection, cancer, or acute coronary syndrome, within the past 3 months, or (ii) serious organ damage defined as cirrhosis, liver cell damage with aspartate aminotransferase or alanine aminotransferase increased ≥ 3 times the upper limit of normal, symptoms of heart failure, impaired renal function (serum creatinine levels >1.7 mg/dL for men and >1.5 mg/dL for women), estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m², or (iii) a history of partial or total gastrectomy or pancreatectomy or colectomy. Patients were also excluded from the study if they were diagnosed with (i) gastrointestinal malabsorption disorders, such as chronic colitis or irritable bowel syndrome, or (ii) pernicious anemia or other hematological disorders. Finally, patients were excluded from the study if they were currently on vitamin B12 or multivitamin replacement, or they had been treated with proton-pump inhibitors or H2-receptor antagonists for at least 12 months consecutively, or they had followed a vegetarian/vegan diet for 3 years.

All the selected patients gave their informed written consent upon participating in the study. Baseline characteristics of the patients were recorded (age, gender, weight, height,

body mass index (BMI), blood pressure, waist and hip circumference, duration of type 2 diabetes, duration of metformin use, and metformin dose). Hypertension was identified based on the criteria of the American College of Cardiology/American Heart Association Task Force in 2017[22], or if the patients were currently on antihypertensive drugs. Diabetic peripheral neuropathies were diagnosed based on patient history or the diagnostic criteria of the Toronto Diabetic Neuropathy Expert Group[23]. The metformin usage index (MUI) was calculated by multiplying the daily metformin dose (mg) by usage duration (year) and dividing by 1000[24].

2. Paraclinical measurements

Fasting blood samples for all the participants were sent to the central laboratory of the Department of Biochemistry, Cho Ray Hospital. Vitamin B12 and folic acid levels were measured using an Architect i2000SR Immunoassay analyzer (Abbott Laboratories, Chicago, USA). Vitamin B12 deficiency was defined as a vitamin B12 level < 300pg/ml with a normal level of folic acid (≥ 4ng/ml). An ADVIA 1800 Chemistry System (Siemens Healthineers, Erlangen, Germany) was used to measure other biochemical parameters, including fasting plasma glucose, creatinine, AST, ALT, and lipid profile. HbA1c was measured using a G8 HPLC analyzer (Tosoh Corporation, Tokyo, Japan). Total blood count was obtained using a DxH 600 Hematology Analyzer (Beckman Coulter).

3. Statistical analysis

Continuous variables were represented either as means ± standard deviation (SD) or as medians with interquartile ranges (IQR), depending on their distribution. These variables were compared using either T-tests or Mann-Whitney U tests. Qualitative variables were characterized by frequencies and counts. Chi-square tests were used to compare categorical variables between groups. To investigate the associations between vitamin B12 deficiency and patients' characteristics, univariate and multivariate logistic regression was applied to calculate odds ratios (OR) and 95% confidence intervals (CI). A p-value of equal or less than 0.05 was considered statistically significant in all analyses. Data analysis was

performed using SPSS Statistics for Windows version 20.0 (IBM Corporation, Armonk, NY, USA)

RESULTS

A total of 145 patients participated in this study. The baseline characteristics of participants are described in Table 1. Briefly, the mean age of the studied population was 61.5, with 51.7% females. The median duration of metformin use was 10 years, the median metformin dose was 1700 mg, and the mean level of HbA1c was 8.1%. The rate of vitamin B12 deficiency was 22.1% (32 out of 145 participants). We could not detect any cases of macrocytic anemia.

There were no differences between the groups with and without vitamin B12 deficiency in terms of age, gender, BMI, hemoglobin level, MCV, creatinine level, and lipid profile. Patients with vitamin B12 deficiency had significantly longer duration of type 2 diabetes, longer duration of metformin use, higher metformin dose, and lower HbA1c levels than patients without vitamin B12 deficiency.

Univariate logistic analyses also showed that hypertension, duration of metformin use, metformin dose, HbA1c level, and MUI were statistically associated with vitamin B12 deficiency in the studied population (Table 2). Notably, a duration of metformin use > median (10 years), metformin dose > median dose (1700 mg/day), and MUI ≥ 10 were strongly associated with vitamin B12 deficiency with OR (95% CI) = 8.539 (2.459–29.648), 4.942 (1.777–13.749), and 5.095 (1.459–17.788), respectively. After adjusting for covariates using continuous and binomial variable models, HbA1c levels, duration of metformin use, and metformin dose were still independently associated with vitamin B12 deficiency (Table 3).

DISCUSSION

In our study, the vitamin B12 deficiency rate was 22.1% in type 2 diabetic Vietnamese patients treated with metformin for at least 3 months. This deficiency rate may vary between countries and ethnicities, and previous studies have reported a wide range of 4.3% to 30% for patients undergoing metformin treatment[21,25–27]. Our study showed similar results to the study by Kim et al. at Yongin Severance Hospital, South Korea, and Gao et al. at 12 tertiary hospitals in China[28,29]. This similarity may reflect consistency between the studies in average age, duration of type 2 diabetes, duration of metformin use, and metformin dosage. However, our results are higher than those of Ko et al. in South Korea, who found a vitamin B12 deficiency rate of 9.5% with a cut-off of vitamin B12 levels ≤ 300 pg/mL and no folate deficiency (folate > 4 ng/mL)[21]. These differences in vitamin B12 deficiency rates between studies may come from differences in testing methods, patient characteristics, and the duration and dosage of metformin prescribed. Except for similar average ages, our patients' duration of type 2 diabetes, duration of metformin use, and average metformin dosage were all higher than those in Ko's study, which partly explains why the vitamin B12 deficiency rate in our study is higher. More importantly, the subjects in our study had a longer duration of type 2 diabetes, longer duration of metformin use, and a higher prescribed metformin dose compared to the patients in Ko's study[21].

Compared with a domestic study in Vietnam by Huynh et al. conducted at Vinmec Central Park Hospital, Ho Chi Minh City in 2023, the vitamin B12 deficiency rate in our study is higher[20]. This difference can be explained by differences in the characteristics of the subjects and the research context. Huynh's study mainly focused on patients undergoing health checks at a private hospital. These are usually patients with better economic conditions, better access to high quality medical services, and who potentially pay more attention to their diet. Thus, the vitamin B12 deficiency rate in their study is lower. Moreover, the patients in Huynh's study were younger, had lower metformin doses, and a shorter duration of metformin treatment. In contrast, our study was conducted at Cho Ray Hospital, a large tertiary hospital that receives patients with complex and more severe

medical conditions. Therefore, the patients in our study had a longer disease duration, longer duration of metformin use, higher metformin dose, and a higher rate of vitamin B12 deficiency.

Our study found that metformin dose and duration of metformin use were statistically associated with vitamin B12 deficiency. These findings are similar to previous studies and emphasize again the important role of vitamin B12 screening in patients with long-term use and/or high doses of metformin[28,30]. In addition, MUI ≥ 10 was found to be an independent factor that is statistically associated with vitamin B12 deficiency, suggesting that MUI can be used as a reliable and alternative parameter to decide when to screen for vitamin B12 deficiency rather than using duration of metformin use or metformin dose alone. We could not use a cut-off value of 5 years for the duration of metformin use and 5 for MUI because the number of participants with the duration of metformin use of less than 5 years was small (only 21 out of 145 cases), leading to underpowered statistical analyses. Interestingly, lower HbA1c was statistically associated with vitamin B12 deficiency in this study. This outcome may result from the fact that patients achieving lower HbA1c were intensively treated with higher dose of metformin.

Our study has several limitations. First, we did not measure methylmalonic acid or homocysteine levels; these markers reflect early vitamin B12 deficiency in tissues. Second, data were collected in a single center and may not represent the landscape of type 2 diabetic patients in the southern region of Vietnam.

In conclusion, our study showed that the rate of vitamin B12 deficiency was up to 22.1% in type 2 diabetic patients with a median of 10 years of metformin use and 1700 mg of metformin dose. This rate presents a warning and requires routine screening and replacement of vitamin B12 in metformin-treated patients. Further multi-center studies are necessary to understand the landscape of vitamin B12 deficiency in patients treated with metformin in Vietnam.

Funding: This study was performed without any specific funding.

Data availability statement: The data that support the findings of this study are available within the article text and tables.

Ethical approval: This study was approved by the Ethics Committee for Medical Research of Cho Ray Hospital (approval number 1711/CN-HĐĐĐ).

Conflict of interest: The authors of this work have nothing to disclose.

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314 Table 1. Baseline characteristics of participants.

Characteristics	Total (N=145)	No vitamin B12 deficiency (N=113)	Vitamin B12 deficiency (N=32)	p-value
Age (years) Mean ± SD	61.5 ± 11.0	60.6 ± 11.0	64.5 ± 10.7	0.075
Gender Female, N (%) Male, N (%)	 75 (51.7) 70 (48.3)	 59 (52.5) 54 (47.8)	 16 (50) 16 (50)	0.844
BMI (kg/m^2) Mean ± SD	22.9 ± 1.9	22.9 ± 2.1	22.8 ± 1.5	0.755
Hypertension Yes, N (%) No, N (%)	 105 (61.9) 40 (38.1)	 77 (68.1) 36 (31.9)	 28 (87.5) 4 (12.5)	**0.031**
Diabetic peripheral neuropathy Yes, N (%) No, N (%)	 54 (37.2) 91 (62.8)	 42 (37.2) 71 (62.8)	 12 (37.5) 20 (62.5)	0.972
Diabetes duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	**0.029**
Metformin use duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	**0.044**
Metformin dose (mg) Median [Q1-Q3]	1700.0 [1000.0 – 1850.0]	1700.0 [1000.0 – 1700.0]	1700.0 [1700.0 – 2000.0]	**0.001**
Hb (g/dl) Mean ± SD	13.6 ± 1.6	13.6 ± 1.6	13.4 ± 1.5	0.674
MCV (fL) Mean ± SD	87.6 ± 6.6	87.1 ± 7.0	89.5 ± 5.1	0.071
Fasting plasma glucose (mg/dl) Mean ± SD	158.4 ± 72.1	162.4 ± 77.2	144.2 ± 48.2	0.208
HbA1c (%) Mean ± SD	8.1 ± 1.9	8.3 ± 2.0	7.4 ± 1.6	**0.021**

Creatinine (mg/dl) Median [Q1-Q3]	0.9 [0.7 – 1.0]	0.9 [0.7 – 1.0]	0.9 [0.8 – 1.0]	0.274
Total cholesterol (mmol/l) Median [Q1-Q3]	146.0 [114.0 – 180.0]	148.0 [114.5 – 181.5]	138.0 [107.3 – 164.5]	0.292
Triglyceride (mmol/l) Median [Q1-Q3]	163.0 [109.5 – 238.0]	163.0 [107.0 – 231.0]	162.0 [131.8 – 307.3]	0.166
HDL-C (mmol/l) Median [Q1-Q3]	44.0 [38.0 – 51.0]	44.0 [39.0 – 52.0]	44.0 [35.0 – 49.0]	0.425
LDL-C (mmol/l) Median [Q1-Q3]	75.0 [49.5 – 107.5]	77.0 [53.8 – 112.5]	64.2 [48.2 – 94.9]	0.242
Vitamin B12 level (pg/ml) Median [Q1-Q3]	478.0 [323.0 – 608.0]	514.0 [416.0 – 667.5]	233.0 [196.5 – 273.8]	**< 0.001**
Folate level (µmol/mL) Mean ± SD	11.5 ± 3.5	11.6 ± 3.5	11.1 ± 3.5	0.457

BMI: body mass index, Hb: hemoglobin, MCV: mean cell volume, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol.

Table 2. Univariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Variables	OR (95% CI)	p-value
Age	1.034 (0.996 – 1.074)	0.077
Gender	1.093 (0.498 – 2.396)	0.825
BMI	0.967 (0.786 – 1.190)	0.753
Hypertension	3.268 (1.068 – 10.000)	**0.038**
Duration of diabetes	1.047 (0.989 – 1.108)	0.115
Duration of metformin use	1.043 (0.985 – 1.103)	0.147
Long-term use of metformin (Duration > median duration)	8.539 (2.459 – 29.648)	**< 0.001**
Metformin dose	1.002 (1.001 – 1.003)	**0.002**
Metformin dose > median dose	4.942 (1.777 – 13.749)	**0.002**
Hb	0.949 (0.744 – 1.210)	0.671
MCV	1.068 (0.993 – 1.148)	0.075
Fasting plasma glucose	0.995 (0.988 – 1.003)	0.210
HbA1c	0.733 (0.557 – 0.964)	**0.026**
MUI	1.043 (1.009 – 1.078)	**0.014**
MUI ≥ 10	5.095 (1.459 – 17.788)	**0.011**

BMI: body mass index, Hb: hemoglobin, MCV: mean cell volume, MUI: metformin usage index.

Table 3. Multivariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Model	Variables	OR (95% CI)	p-value
Model 1	HbA1c	0.624 (0.455 – 0.856)	0.003
	Duration of metformin use	1.076 (1.005 – 1.151)	0.037
	Metformin dose	1.002 (1.001 – 1.004)	0.002
Model 2	HbA1c	0.631 (0.460 – 0.865)	0.004
	Long-term use of metformin (Duration > median duration)	10.078 (2.729 – 37.219)	0.001
	Metformin dose > median dose	4.429 (1.475 – 13.303)	0.008
Model 3	HbA1c	0.647 (0.480 – 0.874)	0.005
	MUI ≥ 10	7.644 (2.109 – 27.707)	0.002

MUI: metformin usage index.

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ASSOCIATION BETWEEN VITAMIN B12 DEFICIENCY AND METFORMIN USE IN TYPE 2 DIABETIC PATIENTS: A CROSS-SECTIONAL STUDY IN A SOUTH VIETNAM TERTIARY HOSPITAL

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ABSTRACT

Objectives: Vitamin B12 deficiency is a common and neglected adverse effect experienced by type 2 diabetic patients treated with metformin. A lack of vitamin B12 may have severe consequences, such as fatigue, macrocytic anemia, and worsened peripheral diabetic neuropathy. This study aims to identify the rate of vitamin B12 deficiency and the associated factors in type 2 diabetic patients treated with metformin.

Design: cross-sectional study.

Setting and participants: This study involved 145 type 2 diabetic patients treated with metformin in Cho Ray hospital, a tertiary healthcare center in the South Vietnam. The levels of vitamin B12 and associated clinical and paraclinical parameters were measured and collected.

Results: The study population's mean age was 61.5, of which 51.7% were female. The median duration of metformin use was 10 years, and the median metformin dose was 1700 mg. The vitamin B12 deficiency rate was 22.1%. HbA1c, duration of metformin use, and metformin dose were independent factors statistically associated with vitamin B12 deficiency.

Conclusions: This study identified the rate of vitamin B12 deficiency and associated factors in type 2 diabetic patients treated with metformin. These findings can be helpful in screening patients and replacing vitamin B12 in high risk populations with vitamin B12 deficiency.

Keywords: metformin; vitamin B12 deficiency; type 2 diabetes mellitus; Vietnam

STRENGTHS AND LIMITATIONS OF THIS STUDY:

- This study investigated the vitamin B12 deficiency rate and its associated factors in type 2 diabetic Vietnamese patients treated with metformin, a population with very limited data.

- The levels of methylmalonic acid and homocysteine were not measured in this study, these markers reflect early vitamin B12 deficiency in tissues.

- This is a single center, cross-sectional study, therefore, the results may not represent the prevalence of vitamin B12 deficiency in type 2 diabetic patients treated with metformin in the southern region of Vietnam.

INTRODUCTION

Metformin belongs to the biguanide class and is synthesized from galegine, an isoprenyl derivative of guanidine found in the perennial herb French lilac (*Galega officinalis*)[1]. Metformin is recommended in most treatment guidelines as the first-line drug for the management of type 2 diabetes, based on its effectiveness in blood glucose control, low cost, safety, and cardiovascular benefits, as demonstrated by the UK Prospective Diabetes Study[2,3].

The drug's effectiveness is dose-dependent, and the mechanism of action of metformin is not fully understood. Extensive evidence obtained in clinical studies and animal models suggests that the main function of metformin in regulating glucose homeostasis is to inhibit hepatic glucose production through the regulation of hepatic gluconeogenesis and glycogenolysis[4,5]. Metformin also improves cellular sensitivity to insulin, thereby enhancing peripheral glucose uptake, mainly in skeletal muscle, and significantly reducing fasting plasma insulin levels[6]. Improvements in insulin sensitivity with metformin may be due to its effects on insulin receptor expression and tyrosine kinase activity.

The most common adverse effects of metformin are gastrointestinal symptoms, such as diarrhea, nausea, and/or abdominal discomfort[7]. These symptoms are usually mild, transient, and dose-dependent. A rare but more concerning adverse effect of biguanides is lactic acidosis. Risk factors for metformin-induced lactic acidosis include conditions that increase lactate production, such as severe infections, severe liver or kidney disease, recent surgery, and any condition causing hypoxia or decreased tissue perfusion[8,9].

In 1969, Berchtold et al. first reported vitamin B12 deficiency in patients using metformin, resulting from decreased gastrointestinal vitamin B12 absorption[10]. This effect can occur as early as the third month of using metformin. Several mechanisms have been proposed to explain the effect of metformin, including changes in small intestinal motility leading to overgrowth of intestinal bacteria, competitive inhibition and inactivation of vitamin B12 absorption capacity, alteration of intrinsic factor levels, and interaction with the

intracellular cubilin receptor[11,12]. Of these, the most widely accepted mechanism is the inhibition of the calcium-dependent binding of the B12-intrinsic factor complex to the cubilin receptor in the ileum. The HOME study is one of the first clinical trials that confirmed the causative effect of vitamin B12 deficiency by metformin. The results showed that metformin usage at a dose of 850 mg three times daily reduces vitamin B12 levels by 19% compared to a placebo[13].

Type 2 diabetes mellitus is a complicated, heterogeneous, and multifactorial disease of which treatment often requires the combination of metformin and other medications[14–19], however, there are few studies on vitamin B12 deficiency related to metformin use in Vietnam. In 2023, Huynh et al. conducted a cross-sectional study on the prevalence of vitamin B12 deficiency in type 2 diabetic patients using metformin at the Vinmec Central Park Hospital, a private outpatient clinic in Ho Chi Minh City[20]. Of the 156 type 2 diabetic patients treated with metformin, the rate of vitamin B12 deficiency was 18.6%. Vitamin B12 deficiency may differ depending on region, diet, and studied population; this study set out to investigate the rate of vitamin B12 deficiency and its associated factors in type 2 diabetic patients treated with metformin in Cho Ray Hospital, one of the largest tertiary hospitals in Vietnam.

MATERIALS AND METHODS

1. Participant recruitment

The protocol for this cross-sectional study was approved by the Ethical Committee of Cho Ray Hospital (approval number 1711/CN-HĐĐĐ). A sample size calculation was performed to estimate the rate of vitamin B12 deficiency in the target population using the formula below:

$$N = Z^2_{(1-\frac{\alpha}{2})} \frac{p(1-p)}{d^2}$$

In which, $Z = 1.96$, p = 0.095 (based on a similar study by Ko et al.[21]), and d = 0.05. The estimated patient number was 132. Recruitment took place from March to August 2024. The main inclusion criteria were type 2 diabetic patients treated with metformin for at least 3 months with unchanged dose in the last 3 months. Patients were excluded from the study if they had (i) a recent acute illness, such as severe infection, cancer, or acute coronary syndrome, within the past 3 months, or (ii) serious organ damage defined as cirrhosis, liver cell damage with aspartate aminotransferase or alanine aminotransferase increased ≥ 3 times the upper limit of normal, symptoms of heart failure, impaired renal function (serum creatinine levels >1.7 mg/dL for men and >1.5 mg/dL for women), estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m², or (iii) a history of partial or total gastrectomy or pancreatectomy or colectomy. Patients were also excluded from the study if they were diagnosed with (i) gastrointestinal malabsorption disorders, such as chronic colitis or irritable bowel syndrome, or (ii) pernicious anemia or other hematological disorders. Finally, patients were excluded from the study if they were currently on vitamin B12 or multivitamin replacement, or they had been treated with proton-pump inhibitors or H2-receptor antagonists for at least 12 months consecutively, or they had followed a vegetarian/vegan diet for 3 years.

All the selected patients gave their informed written consent upon participating in the study. Baseline characteristics of the patients were recorded (age, gender, weight, height,

body mass index (BMI), blood pressure, waist and hip circumference, duration of type 2 diabetes, duration of metformin use, and metformin dose). Hypertension was identified based on the criteria of the American College of Cardiology/American Heart Association Task Force in 2017[22], or if the patients were currently on antihypertensive drugs. Diabetic peripheral neuropathies were diagnosed based on patient history or the diagnostic criteria of the Toronto Diabetic Neuropathy Expert Group[23]. The metformin usage index (MUI) was calculated by multiplying the daily metformin dose (mg) by usage duration (year) and dividing by 1000[24].

All the patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research

2. Paraclinical measurements

Fasting blood samples for all the participants were sent to the central laboratory of the Department of Biochemistry, Cho Ray Hospital. Vitamin B12 and folic acid levels were measured using an Architect i2000SR Immunoassay analyzer (Abbott Laboratories, Chicago, USA). Vitamin B12 deficiency was defined as a vitamin B12 level < 300pg/ml with a normal level of folic acid (≥ 4ng/ml). An ADVIA 1800 Chemistry System (Siemens Healthineers, Erlangen, Germany) was used to measure other biochemical parameters, including fasting plasma glucose, creatinine, AST, ALT, and lipid profile. HbA1c was measured using a G8 HPLC analyzer (Tosoh Corporation, Tokyo, Japan). Total blood count was obtained using a DxH 600 Hematology Analyzer (Beckman Coulter).

3. Statistical analysis

Continuous variables were represented either as means ± standard deviation (SD) or as medians with interquartile ranges (IQR), depending on their distribution. These variables were compared using either T-tests or Mann-Whitney U tests. Qualitative variables were characterized by frequencies and counts. Chi-square tests were used to compare categorical variables between groups. To investigate the associations between vitamin B12 deficiency and patients' characteristics, univariate and multivariate logistic regression was applied to

calculate odds ratios (OR) and 95% confidence intervals (CI). A p-value of equal or less than 0.05 was considered statistically significant in all analyses. Data analysis was performed using SPSS Statistics for Windows version 20.0 (IBM Corporation, Armonk, NY, USA)

RESULTS

A total of 145 patients participated in this study. The baseline characteristics of participants are described in Table 1. Briefly, the mean age of the studied population was 61.5, with 51.7% females. The median duration of metformin use was 10 years, the median metformin dose was 1700 mg, and the mean level of HbA1c was 8.1%. The rate of vitamin B12 deficiency was 22.1% (32 out of 145 participants). We could not detect any cases of macrocytic anemia.

There were no differences between the groups with and without vitamin B12 deficiency in terms of age, gender, BMI, hemoglobin level, MCV, creatinine level, and lipid profile. Patients with vitamin B12 deficiency had significantly longer duration of type 2 diabetes, longer duration of metformin use, higher metformin dose, and lower HbA1c levels than patients without vitamin B12 deficiency. The medications used for type 2 diabetes treatment including insulin, sulfonyl urea, dipeptidyl peptidase 4 inhibitor (DDP4i), and sodium-glucose cotransporter 2 inhibitor (SGLT2i) were not statistically different between the groups with and without vitamin B12 deficiency.

Univariate logistic analyses also showed that hypertension, duration of metformin use, metformin dose, HbA1c level, and MUI were statistically associated with vitamin B12 deficiency in the studied population (Table 2). Notably, a duration of metformin use > median (10 years), metformin dose > median dose (1700 mg/day), and MUI ≥ 10 were strongly associated with vitamin B12 deficiency with OR (95% CI) = 8.539 (2.459–29.648), 4.942 (1.777–13.749), and 5.095 (1.459–17.788), respectively. After adjusting for covariates using continuous and binomial variable models, HbA1c levels, duration of metformin use, and metformin dose were still independently associated with vitamin B12 deficiency (Table 3).

DISCUSSION

In our study, the vitamin B12 deficiency rate was 22.1% in type 2 diabetic Vietnamese patients treated with metformin for at least 3 months. This deficiency rate may vary between countries and ethnicities, and previous studies have reported a wide range of 4.3% to 30% for patients undergoing metformin treatment[21,25–27]. Our study showed similar results to the study by Kim et al. at Yongin Severance Hospital, South Korea, and Gao et al. at 12 tertiary hospitals in China[28,29]. This similarity may reflect consistency between the studies in average age, duration of type 2 diabetes, duration of metformin use, and metformin dosage. However, our results are higher than those of Ko et al. in South Korea, who found a vitamin B12 deficiency rate of 9.5% with a cut-off of vitamin B12 levels ≤ 300 pg/mL and no folate deficiency (folate > 4 ng/mL)[21]. These differences in vitamin B12 deficiency rates between studies may come from differences in testing methods, patient characteristics, and the duration and dosage of metformin prescribed. Except for similar average ages, our patients' duration of type 2 diabetes, duration of metformin use, and average metformin dosage were all higher than those in Ko's study, which partly explains why the vitamin B12 deficiency rate in our study is higher. More importantly, the subjects in our study had a longer duration of type 2 diabetes, longer duration of metformin use, and a higher prescribed metformin dose compared to the patients in Ko's study[21].

Compared with a domestic study in Vietnam by Huynh et al. conducted at Vinmec Central Park Hospital, Ho Chi Minh City in 2023, the vitamin B12 deficiency rate in our study is higher[20]. This difference can be explained by differences in the characteristics of the subjects and the research context. Huynh's study mainly focused on patients undergoing health checks at a private hospital. These are usually patients with better economic conditions, better access to high quality medical services, and who potentially pay more attention to their diet. Thus, the vitamin B12 deficiency rate in their study is lower. Moreover, the patients in Huynh's study were younger, had lower metformin doses, and a shorter duration of metformin treatment. In contrast, our study was conducted at Cho Ray Hospital, a large tertiary hospital that receives patients with complex and more severe

medical conditions. Therefore, the patients in our study had a longer disease duration, longer duration of metformin use, higher metformin dose, and a higher rate of vitamin B12 deficiency.

Our study found that metformin dose and duration of metformin use were statistically associated with vitamin B12 deficiency. These findings are similar to previous studies and emphasize again the important role of vitamin B12 screening in patients with long-term use and/or high doses of metformin[28,30]. In addition, MUI ≥ 10 was found to be an independent factor that is statistically associated with vitamin B12 deficiency, suggesting that MUI can be used as a reliable and alternative parameter to decide when to screen for vitamin B12 deficiency rather than using duration of metformin use or metformin dose alone. We could not use a cut-off value of 5 years for the duration of metformin use and 5 for MUI because the number of participants with the duration of metformin use of less than 5 years was small (only 21 out of 145 cases), leading to underpowered statistical analyses. Interestingly, lower HbA1c was statistically associated with vitamin B12 deficiency in this study. This outcome may result from the fact that patients achieving lower HbA1c were intensively treated with higher dose of metformin.

Our study has several limitations. First, we did not measure methylmalonic acid or homocysteine levels; these markers reflect early vitamin B12 deficiency in tissues. Second, data were collected in a single center and may not represent the landscape of type 2 diabetic patients in the southern region of Vietnam.

In conclusion, our study showed that the rate of vitamin B12 deficiency was up to 22.1% in type 2 diabetic patients with a median of 10 years of metformin use and 1700 mg of metformin dose. This rate presents a warning and requires routine screening and replacement of vitamin B12 in metformin-treated patients. Further multi-center studies are necessary to understand the landscape of vitamin B12 deficiency in patients treated with metformin in Vietnam.

Author Contributions: HP, AN, MD designed the research study. AN recruited the participants for the study. HP and MD analyzed the data. HP, AN, and MD wrote the manuscript. MD is the guarantor.

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Data availability statement: Data supporting the findings of this study are available from the corresponding author [MD] on request. Raw data were generated at Cho Ray hospital, Ho Chi Minh City, Vietnam.

Ethical approval: This study was approved by the Ethics Committee for Medical Research of Cho Ray Hospital (approval number 1711/CN-HĐĐĐ).

Conflict of interest: The authors of this work have nothing to disclose.

Patient and public involvement: Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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336 Table 1. Baseline characteristics of participants.

Characteristics	Total (N=145)	No vitamin B12 deficiency (N=113)	Vitamin B12 deficiency (N=32)	p-value
Age (years) Mean ± SD	61.5 ± 11.0	60.6 ± 11.0	64.5 ± 10.7	0.075
Gender				0.844
Female, N (%)	75 (51.7)	59 (52.5)	16 (50)	
Male, N (%)	70 (48.3)	54 (47.8)	16 (50)	
BMI (kg/m^2) Mean ± SD	22.9 ± 1.9	22.9 ± 2.1	22.8 ± 1.5	0.755
Hypertension				**0.031**
Yes, N (%)	105 (61.9)	77 (68.1)	28 (87.5)	
No, N (%)	40 (38.1)	36 (31.9)	4 (12.5)	
Diabetic peripheral neuropathy				0.972
Yes, N (%)	54 (37.2)	42 (37.2)	12 (37.5)	
No, N (%)	91 (62.8)	71 (62.8)	20 (62.5)	
Diabetes duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	**0.029**
Metformin use duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	**0.044**
Metformin dose (mg) Median [Q1-Q3]	1700.0 [1000.0 – 1850.0]	1700.0 [1000.0 – 1700.0]	1700.0 [1700.0 – 2000.0]	**0.001**
Hb (g/dl) Mean ± SD	13.6 ± 1.6	13.6 ± 1.6	13.4 ± 1.5	0.674
MCV (fL) Mean ± SD	87.6 ± 6.6	87.1 ± 7.0	89.5 ± 5.1	0.071
Fasting plasma glucose (mg/dl) Mean ± SD	158.4 ± 72.1	162.4 ± 77.2	144.2 ± 48.2	0.208
HbA1c (%) Mean ± SD	8.1 ± 1.9	8.3 ± 2.0	7.4 ± 1.6	**0.021**

Creatinine (mg/dl) Median [Q1-Q3]	0.9 [0.7 – 1.0]	0.9 [0.7 – 1.0]	0.9 [0.8 – 1.0]	0.274
Total cholesterol (mmol/l) Median [Q1-Q3]	146.0 [114.0 – 180.0]	148.0 [114.5 – 181.5]	138.0 [107.3 – 164.5]	0.292
Triglyceride (mmol/l) Median [Q1-Q3]	163.0 [109.5 – 238.0]	163.0 [107.0 – 231.0]	162.0 [131.8 – 307.3]	0.166
HDL-C (mmol/l) Median [Q1-Q3]	44.0 [38.0 – 51.0]	44.0 [39.0 – 52.0]	44.0 [35.0 – 49.0]	0.425
LDL-C (mmol/l) Median [Q1-Q3]	75.0 [49.5 – 107.5]	77.0 [53.8 – 112.5]	64.2 [48.2 – 94.9]	0.242
Vitamin B12 level (pg/ml) Median [Q1-Q3]	478.0 [323.0 – 608.0]	514.0 [416.0 – 667.5]	233.0 [196.5 – 273.8]	**< 0.001**
Folate level (µmol/mL) Mean ± SD	11.5 ± 3.5	11.6 ± 3.5	11.1 ± 3.5	0.457
Insulin treatment				0.684
Yes, N (%)	50 (34.5)	38 (33.6)	12 (37.5)	
No, N (%)	95 (65.5)	75 (66.4)	20 (62.5)	
Sulfonyl urea treament				0.370
Yes, N (%)	45 (31.0)	33 (29.2)	12 (37.5)	
No, N (%)	100 (69.0)	80 (70.8)	20 (62.5)	
DPP4i treatment				0.089
Yes, N (%)	116 (80.0)	87 (77.0)	29 (90.6)	
No, N (%)	29 (20.0)	26 (23.0)	3 (9.4)	
SGLT2i treatment				0.161
Yes, N (%)	88 (60.7)	72 (63.7)	16 (50.0)	
No, N (%)	57 (39.3)	41 (36.3)	16 (50.0)	

BMI: body mass index, DPP4i: dipeptidyl peptidase 4 inhibitor, Hb: hemoglobin, MCV: mean cell volume, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, SGLT2i: sodium-glucose cotransporter 2 inhibitor.

Table 2. Univariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Variables	OR (95% CI)	p-value
Age	1.034 (0.996 – 1.074)	0.077
Gender	1.093 (0.498 – 2.396)	0.825
BMI	0.967 (0.786 – 1.190)	0.753
Hypertension	3.268 (1.068 – 10.000)	**0.038**
Duration of diabetes	1.047 (0.989 – 1.108)	0.115
Duration of metformin use	1.043 (0.985 – 1.103)	0.147
Long-term use of metformin (Duration > median duration)	8.539 (2.459 – 29.648)	**< 0.001**
Metformin dose	1.002 (1.001 – 1.003)	**0.002**
Metformin dose > median dose	4.942 (1.777 – 13.749)	**0.002**
Hb	0.949 (0.744 – 1.210)	0.671
MCV	1.068 (0.993 – 1.148)	0.075
Fasting plasma glucose	0.995 (0.988 – 1.003)	0.210
HbA1c	0.733 (0.557 – 0.964)	**0.026**
MUI	1.043 (1.009 – 1.078)	**0.014**
MUI ≥ 10	5.095 (1.459 – 17.788)	**0.011**

BMI: body mass index, Hb: hemoglobin, MCV: mean cell volume, MUI: metformin usage index.

Table 3. Multivariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Model	Variables	OR (95% CI)	p-value
Model 1	HbA1c	0.624 (0.455 – 0.856)	0.003
	Duration of metformin use	1.076 (1.005 – 1.151)	0.037
	Metformin dose	1.002 (1.001 – 1.004)	0.002
Model 2	HbA1c	0.631 (0.460 – 0.865)	0.004
	Long-term use of metformin (Duration > median duration)	10.078 (2.729 – 37.219)	0.001
	Metformin dose > median dose	4.429 (1.475 – 13.303)	0.008
Model 3	HbA1c	0.647 (0.480 – 0.874)	0.005
	MUI ≥ 10	7.644 (2.109 – 27.707)	0.002

MUI: metformin usage index.

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ASSOCIATION BETWEEN VITAMIN B12 DEFICIENCY AND METFORMIN USE IN TYPE 2 DIABETIC PATIENTS: A CROSS-SECTIONAL STUDY IN A SOUTH VIETNAM TERTIARY HOSPITAL

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ABSTRACT

Objectives: Vitamin B12 deficiency is a common and neglected adverse effect experienced by type 2 diabetic patients treated with metformin. A lack of vitamin B12 may have severe consequences, such as fatigue, macrocytic anemia, and worsened peripheral diabetic neuropathy. This study aims to identify the rate of vitamin B12 deficiency and the associated factors in type 2 diabetic patients treated with metformin.

Design: cross-sectional study.

Setting and participants: This study involved 145 type 2 diabetic patients treated with metformin in Cho Ray hospital, a tertiary healthcare center in the South Vietnam. The levels of vitamin B12 and associated clinical and paraclinical parameters were measured and collected.

Results: The study population's mean age was 61.5, of which 51.7% were female. The median duration of metformin use was 10 years, and the median metformin dose was 1700 mg. The vitamin B12 deficiency rate was 22.1%. HbA1c, duration of metformin use, and metformin dose were independent factors statistically associated with vitamin B12 deficiency.

Conclusions: This study identified the rate of vitamin B12 deficiency and associated factors in type 2 diabetic patients treated with metformin. These findings can be helpful in screening patients and replacing vitamin B12 in high risk populations with vitamin B12 deficiency.

Keywords: metformin; vitamin B12 deficiency; type 2 diabetes mellitus; Vietnam

STRENGTHS AND LIMITATIONS OF THIS STUDY:

- This study investigated the vitamin B12 deficiency rate and its associated factors in type 2 diabetic Vietnamese patients treated with metformin, a population with very limited data.

- The levels of methylmalonic acid and homocysteine were not measured in this study, these markers reflect early vitamin B12 deficiency in tissues.

- This is a single center, cross-sectional study, therefore, the results may not represent the prevalence of vitamin B12 deficiency in type 2 diabetic patients treated with metformin in the southern region of Vietnam.

INTRODUCTION

Metformin belongs to the biguanide class and is synthesized from galegine, an isoprenyl derivative of guanidine found in the perennial herb French lilac (*Galega officinalis*)[1]. Metformin is recommended in most treatment guidelines as the first-line drug for the management of type 2 diabetes, based on its effectiveness in blood glucose control, low cost, safety, and cardiovascular benefits, as demonstrated by the UK Prospective Diabetes Study[2,3].

The drug's effectiveness is dose-dependent, and the mechanism of action of metformin is not fully understood. Extensive evidence obtained in clinical studies and animal models suggests that the main function of metformin in regulating glucose homeostasis is to inhibit hepatic glucose production through the regulation of hepatic gluconeogenesis and glycogenolysis[4,5]. Metformin also improves cellular sensitivity to insulin, thereby enhancing peripheral glucose uptake, mainly in skeletal muscle, and significantly reducing fasting plasma insulin levels[6]. Improvements in insulin sensitivity with metformin may be due to its effects on insulin receptor expression and tyrosine kinase activity.

The most common adverse effects of metformin are gastrointestinal symptoms, such as diarrhea, nausea, and/or abdominal discomfort[7]. These symptoms are usually mild, transient, and dose-dependent. A rare but more concerning adverse effect of biguanides is lactic acidosis. Risk factors for metformin-induced lactic acidosis include conditions that increase lactate production, such as severe infections, severe liver or kidney disease, recent surgery, and any condition causing hypoxia or decreased tissue perfusion[8,9].

In 1969, Berchtold et al. first reported vitamin B12 deficiency in patients using metformin, resulting from decreased gastrointestinal vitamin B12 absorption[10]. This effect can occur as early as the third month of using metformin. Several mechanisms have been proposed to explain the effect of metformin, including changes in small intestinal motility leading to overgrowth of intestinal bacteria, competitive inhibition and inactivation of vitamin B12 absorption capacity, alteration of intrinsic factor levels, and interaction with the

intracellular cubilin receptor[11,12]. Of these, the most widely accepted mechanism is the inhibition of the calcium-dependent binding of the B12-intrinsic factor complex to the cubilin receptor in the ileum. The HOME study is one of the first clinical trials that confirmed the causative effect of vitamin B12 deficiency by metformin. The results showed that metformin usage at a dose of 850 mg three times daily reduces vitamin B12 levels by 19% compared to a placebo[13].

Type 2 diabetes mellitus is a complicated, heterogeneous, and multifactorial disease of which treatment often requires the combination of metformin and other medications[14–19], however, there are few studies on vitamin B12 deficiency related to metformin use in Vietnam. In 2023, Huynh et al. conducted a cross-sectional study on the prevalence of vitamin B12 deficiency in type 2 diabetic patients using metformin at the Vinmec Central Park Hospital, a private outpatient clinic in Ho Chi Minh City[20]. Of the 156 type 2 diabetic patients treated with metformin, the rate of vitamin B12 deficiency was 18.6%. Vitamin B12 deficiency may differ depending on region, diet, and studied population; this study set out to investigate the rate of vitamin B12 deficiency and its associated factors in type 2 diabetic patients treated with metformin in Cho Ray Hospital, one of the largest tertiary hospitals in Vietnam.

MATERIALS AND METHODS

1. Participant recruitment

The protocol for this cross-sectional study was approved by the Ethical Committee of Cho Ray Hospital (approval number 1711/CN-HĐĐĐ). A sample size calculation was performed to estimate the rate of vitamin B12 deficiency in the target population using the formula below:

$$N = Z^2_{(1-\frac{\alpha}{2})} \frac{p(1-p)}{d^2}$$

In which, $Z = 1.96$, $p = 0.095$ (based on a similar study by Ko et al.[21]), and $d = 0.05$. The estimated patient number was 132. Recruitment took place from March to August 2024. The main inclusion criteria were type 2 diabetic patients treated with metformin for at least 3 months with unchanged dose in the last 3 months. Patients were excluded from the study if they had (i) a recent acute illness, such as severe infection, cancer, or acute coronary syndrome, within the past 3 months, or (ii) serious organ damage defined as cirrhosis, liver cell damage with aspartate aminotransferase or alanine aminotransferase increased ≥ 3 times the upper limit of normal, symptoms of heart failure, impaired renal function (serum creatinine levels >1.7 mg/dL for men and >1.5 mg/dL for women), estimated glomerular filtration rate (eGFR) < 30 mL/min/1.73 m², or (iii) a history of partial or total gastrectomy or pancreatectomy or colectomy. Patients were also excluded from the study if they were diagnosed with (i) gastrointestinal malabsorption disorders, such as chronic colitis or irritable bowel syndrome, or (ii) pernicious anemia or other hematological disorders. Finally, patients were excluded from the study if they were currently on vitamin B12 or multivitamin replacement, or they had been treated with proton-pump inhibitors or H2-receptor antagonists for at least 12 months consecutively, or they had followed a vegetarian/vegan diet for 3 years.

All the selected patients gave their informed written consent upon participating in the study. Baseline characteristics of the patients were recorded (age, gender, weight, height,

body mass index (BMI), blood pressure, waist and hip circumference, duration of type 2 diabetes, duration of metformin use, and metformin dose). Hypertension was identified based on the criteria of the American College of Cardiology/American Heart Association Task Force in 2017[22], or if the patients were currently on antihypertensive drugs. Diabetic peripheral neuropathies were diagnosed based on patient history or the diagnostic criteria of the Toronto Diabetic Neuropathy Expert Group[23]. The metformin usage index (MUI) was calculated by multiplying the daily metformin dose (mg) by usage duration (year) and dividing by 1000[24].

All the patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research

2. Paraclinical measurements

Fasting blood samples for all the participants were sent to the central laboratory of the Department of Biochemistry, Cho Ray Hospital. Vitamin B12 and folic acid levels were measured using an Architect i2000SR Immunoassay analyzer (Abbott Laboratories, Chicago, USA). Vitamin B12 deficiency was defined as a vitamin B12 level < 300pg/ml with a normal level of folic acid (≥ 4ng/ml). An ADVIA 1800 Chemistry System (Siemens Healthineers, Erlangen, Germany) was used to measure other biochemical parameters, including fasting plasma glucose, creatinine, AST, ALT, and lipid profile. HbA1c was measured using a G8 HPLC analyzer (Tosoh Corporation, Tokyo, Japan). Total blood count was obtained using a DxH 600 Hematology Analyzer (Beckman Coulter).

3. Statistical analysis

Continuous variables were represented either as means ± standard deviation (SD) or as medians with interquartile ranges (IQR), depending on their distribution. These variables were compared using either T-tests or Mann-Whitney U tests. Qualitative variables were characterized by frequencies and counts. Chi-square tests were used to compare categorical variables between groups. To investigate the associations between vitamin B12 deficiency and patients' characteristics, univariate and multivariate logistic regression was applied to

calculate odds ratios (OR) and 95% confidence intervals (CI). A p-value of equal or less than 0.05 was considered statistically significant in all analyses. Data analysis was performed using SPSS Statistics for Windows version 20.0 (IBM Corporation, Armonk, NY, USA)

RESULTS

A total of 145 patients participated in this study. The baseline characteristics of participants are described in Table 1. Briefly, the mean age of the studied population was 61.5, with 51.7% females. The median duration of metformin use was 10 years, the median metformin dose was 1700 mg, and the mean level of HbA1c was 8.1%. The rate of vitamin B12 deficiency was 22.1% (32 out of 145 participants). We could not detect any cases of macrocytic anemia.

There were no differences between the groups with and without vitamin B12 deficiency in terms of age, gender, BMI, hemoglobin level, MCV, creatinine level, and lipid profile. Patients with vitamin B12 deficiency had significantly longer duration of type 2 diabetes, longer duration of metformin use, higher metformin dose, and lower HbA1c levels than patients without vitamin B12 deficiency. The medications used for type 2 diabetes treatment including insulin, sulfonyl urea, dipeptidyl peptidase 4 inhibitor (DDP4i), and sodium-glucose cotransporter 2 inhibitor (SGLT2i) were not statistically different between the groups with and without vitamin B12 deficiency.

Univariate logistic analyses also showed that hypertension, duration of metformin use, metformin dose, HbA1c level, and MUI were statistically associated with vitamin B12 deficiency in the studied population (Table 2). Notably, a duration of metformin use > median (10 years), metformin dose > median dose (1700 mg/day), and MUI ≥ 10 were strongly associated with vitamin B12 deficiency with OR (95% CI) = 8.539 (2.459–29.648), 4.942 (1.777–13.749), and 5.095 (1.459–17.788), respectively. After adjusting for covariates using continuous and binomial variable models, HbA1c levels, duration of metformin use, and metformin dose were still independently associated with vitamin B12 deficiency (Table 3).

DISCUSSION

In our study, the vitamin B12 deficiency rate was 22.1% in type 2 diabetic Vietnamese patients treated with metformin for at least 3 months. This deficiency rate may vary between countries and ethnicities, and previous studies have reported a wide range of 4.3% to 30% for patients undergoing metformin treatment[21,25–27]. Our study showed similar results to the study by Kim et al. at Yongin Severance Hospital, South Korea, and Gao et al. at 12 tertiary hospitals in China[28,29]. This similarity may reflect consistency between the studies in average age, duration of type 2 diabetes, duration of metformin use, and metformin dosage. However, our results are higher than those of Ko et al. in South Korea, who found a vitamin B12 deficiency rate of 9.5% with a cut-off of vitamin B12 levels ≤ 300 pg/mL and no folate deficiency (folate > 4 ng/mL)[21]. These differences in vitamin B12 deficiency rates between studies may come from differences in testing methods, patient characteristics, and the duration and dosage of metformin prescribed. Except for similar average ages, our patients' duration of type 2 diabetes, duration of metformin use, and average metformin dosage were all higher than those in Ko's study, which partly explains why the vitamin B12 deficiency rate in our study is higher. More importantly, the subjects in our study had a longer duration of type 2 diabetes, longer duration of metformin use, and a higher prescribed metformin dose compared to the patients in Ko's study[21]. There were several reasons why vitamin B12 deficiency rate was 22.1% but did not occur in all the participants. First, not all the participants were treated with metformin for four consecutive years and/or with high metformin dose. Second, the exact mechanisms of vitamin B12 deficiency in patients treated with metformin remained elusive, and who will absolutely develop vitamin B12 deficiency is still an unanswered question. Therefore, current American Diabetes Association guidelines recommend screening for high-risk patients and treating if they have vitamin B12 deficiency rather than treating all patients with metformin use for four consecutive years and more[30].

Compared with a domestic study in Vietnam by Huynh et al. conducted at Vinmec Central Park Hospital, Ho Chi Minh City in 2023, the vitamin B12 deficiency rate in our study is

higher[20]. This difference can be explained by differences in the characteristics of the subjects and the research context. Huynh's study mainly focused on patients undergoing health checks at a private hospital. These are usually patients with better economic conditions, better access to high quality medical services, and who potentially pay more attention to their diet. Thus, the vitamin B12 deficiency rate in their study is lower. Moreover, the patients in Huynh's study were younger, had lower metformin doses, and a shorter duration of metformin treatment. In contrast, our study was conducted at Cho Ray Hospital, a large tertiary hospital that receives patients with complex and more severe medical conditions. Therefore, the patients in our study had a longer disease duration, longer duration of metformin use, higher metformin dose, and a higher rate of vitamin B12 deficiency.

Our study found that metformin dose and duration of metformin use were statistically associated with vitamin B12 deficiency. These findings are similar to previous studies and emphasize again the important role of vitamin B12 screening in patients with long-term use and/or high doses of metformin[28,31]. In addition, MUI ≥ 10 was found to be an independent factor that is statistically associated with vitamin B12 deficiency, suggesting that MUI can be used as a reliable and alternative parameter to decide when to screen for vitamin B12 deficiency rather than using duration of metformin use or metformin dose alone. We could not use a cut-off value of 5 years for the duration of metformin use and 5 for MUI because the number of participants with the duration of metformin use of less than 5 years was small (only 21 out of 145 cases), leading to underpowered statistical analyses. Interestingly, lower HbA1c was statistically associated with vitamin B12 deficiency in this study. This outcome may result from the fact that patients achieving lower HbA1c were intensively treated with higher dose of metformin.

Our study has several limitations. First, we did not measure methylmalonic acid or homocysteine levels; these markers reflect early vitamin B12 deficiency in tissues. Second, the sample size was small, and all the data were collected in a single center, therefore, the

reported results may not represent the landscape of type 2 diabetic patients in the southern region of Vietnam.

In conclusion, our study showed that the rate of vitamin B12 deficiency was up to 22.1% in type 2 diabetic patients with a median of 10 years of metformin use and 1700 mg of metformin dose. This rate presents a warning and requires routine screening and replacement of vitamin B12 in metformin-treated patients. Further multi-center studies are necessary to understand the landscape of vitamin B12 deficiency in patients treated with metformin in Vietnam.

Author Contributions: HP, AN, MD designed the research study. AN recruited the participants for the study. HP and MD analyzed the data. HP, AN, and MD wrote the manuscript. MD is the guarantor.

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Data availability statement: Data supporting the findings of this study are available from the corresponding author [MD] on request. Raw data were generated at Cho Ray hospital, Ho Chi Minh City, Vietnam.

Ethical approval: This study was approved by the Ethics Committee for Medical Research of Cho Ray Hospital (approval number 1711/CN-HĐĐĐ).

Conflict of interest: The authors of this work have nothing to disclose.

Patient and public involvement: Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

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348 Table 1. Baseline characteristics of participants.

Characteristics	Total (N=145)	No vitamin B12 deficiency (N=113)	Vitamin B12 deficiency (N=32)	p-value
Age (years) Mean ± SD	61.5 ± 11.0	60.6 ± 11.0	64.5 ± 10.7	0.075
Gender Female, N (%) Male, N (%)	 75 (51.7) 70 (48.3)	 59 (52.5) 54 (47.8)	 16 (50) 16 (50)	0.844
BMI (kg/m^2) Mean ± SD	22.9 ± 1.9	22.9 ± 2.1	22.8 ± 1.5	0.755
Hypertension Yes, N (%) No, N (%)	 105 (61.9) 40 (38.1)	 77 (68.1) 36 (31.9)	 28 (87.5) 4 (12.5)	**0.031**
Diabetic peripheral neuropathy Yes, N (%) No, N (%)	 54 (37.2) 91 (62.8)	 42 (37.2) 71 (62.8)	 12 (37.5) 20 (62.5)	0.972
Diabetes duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	**0.029**
Metformin use duration (years) Median [Q1-Q3]	10.0 [6.0 – 10.0]	10.0 [5.5 – 15.0]	12.0 [10.0 – 15.0]	**0.044**
Metformin dose (mg) Median [Q1-Q3]	1700.0 [1000.0 – 1850.0]	1700.0 [1000.0 – 1700.0]	1700.0 [1700.0 – 2000.0]	**0.001**
Hb (g/dl) Mean ± SD	13.6 ± 1.6	13.6 ± 1.6	13.4 ± 1.5	0.674
MCV (fL) Mean ± SD	87.6 ± 6.6	87.1 ± 7.0	89.5 ± 5.1	0.071
Fasting plasma glucose (mg/dl) Mean ± SD	158.4 ± 72.1	162.4 ± 77.2	144.2 ± 48.2	0.208
HbA1c (%) Mean ± SD	8.1 ± 1.9	8.3 ± 2.0	7.4 ± 1.6	**0.021**

Creatinine (mg/dl) Median [Q1-Q3]	0.9 [0.7 – 1.0]	0.9 [0.7 – 1.0]	0.9 [0.8 – 1.0]	0.274
Total cholesterol (mmol/l) Median [Q1-Q3]	146.0 [114.0 – 180.0]	148.0 [114.5 – 181.5]	138.0 [107.3 – 164.5]	0.292
Triglyceride (mmol/l) Median [Q1-Q3]	163.0 [109.5 – 238.0]	163.0 [107.0 – 231.0]	162.0 [131.8 – 307.3]	0.166
HDL-C (mmol/l) Median [Q1-Q3]	44.0 [38.0 – 51.0]	44.0 [39.0 – 52.0]	44.0 [35.0 – 49.0]	0.425
LDL-C (mmol/l) Median [Q1-Q3]	75.0 [49.5 – 107.5]	77.0 [53.8 – 112.5]	64.2 [48.2 – 94.9]	0.242
Vitamin B12 level (pg/ml) Median [Q1-Q3]	478.0 [323.0 – 608.0]	514.0 [416.0 – 667.5]	233.0 [196.5 – 273.8]	**< 0.001**
Folate level (µmol/mL) Mean ± SD	11.5 ± 3.5	11.6 ± 3.5	11.1 ± 3.5	0.457
Insulin treatment				0.684
Yes, N (%)	50 (34.5)	38 (33.6)	12 (37.5)	
No, N (%)	95 (65.5)	75 (66.4)	20 (62.5)	
Sulfonyl urea treament				0.370
Yes, N (%)	45 (31.0)	33 (29.2)	12 (37.5)	
No, N (%)	100 (69.0)	80 (70.8)	20 (62.5)	
DPP4i treatment				0.089
Yes, N (%)	116 (80.0)	87 (77.0)	29 (90.6)	
No, N (%)	29 (20.0)	26 (23.0)	3 (9.4)	
SGLT2i treatment				0.161
Yes, N (%)	88 (60.7)	72 (63.7)	16 (50.0)	
No, N (%)	57 (39.3)	41 (36.3)	16 (50.0)	

BMI: body mass index, DPP4i: dipeptidyl peptidase 4 inhibitor, Hb: hemoglobin, MCV: mean cell volume, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol, SGLT2i: sodium-glucose cotransporter 2 inhibitor.

Table 2. Univariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Variables	OR (95% CI)	p-value
Age	1.034 (0.996 – 1.074)	0.077
Gender	1.093 (0.498 – 2.396)	0.825
BMI	0.967 (0.786 – 1.190)	0.753
Hypertension	3.268 (1.068 – 10.000)	**0.038**
Duration of diabetes	1.047 (0.989 – 1.108)	0.115
Duration of metformin use	1.043 (0.985 – 1.103)	0.147
Long-term use of metformin (Duration > median duration)	8.539 (2.459 – 29.648)	**< 0.001**
Metformin dose	1.002 (1.001 – 1.003)	**0.002**
Metformin dose > median dose	4.942 (1.777 – 13.749)	**0.002**
Hb	0.949 (0.744 – 1.210)	0.671
MCV	1.068 (0.993 – 1.148)	0.075
Fasting plasma glucose	0.995 (0.988 – 1.003)	0.210
HbA1c	0.733 (0.557 – 0.964)	**0.026**
MUI	1.043 (1.009 – 1.078)	**0.014**
MUI ≥ 10	5.095 (1.459 – 17.788)	**0.011**

BMI: body mass index, Hb: hemoglobin, MCV: mean cell volume, MUI: metformin usage index.

Table 3. Multivariate logistic regression analysis of factors associated with vitamin B12 deficiency.

Model	Variables	OR (95% CI)	p-value
Model 1	HbA1c	0.624 (0.455 – 0.856)	0.003
	Duration of metformin use	1.076 (1.005 – 1.151)	0.037
	Metformin dose	1.002 (1.001 – 1.004)	0.002
Model 2	HbA1c	0.631 (0.460 – 0.865)	0.004
	Long-term use of metformin (Duration > median duration)	10.078 (2.729 – 37.219)	0.001
	Metformin dose > median dose	4.429 (1.475 – 13.303)	0.008
Model 3	HbA1c	0.647 (0.480 – 0.874)	0.005
	MUI ≥ 10	7.644 (2.109 – 27.707)	0.002

MUI: metformin usage index.