Prevalence of Vitamin B12 Deficiency and its Associated Factors among Patients with Type 2 Diabetes Mellitus on Metformin from a District in Malaysia

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Abstract

Introduction. Vitamin B12 deficiency is more common among metformin-treated subjects although the prevalence is variable. Many factors have been associated with this. The aim of this study is to determine the prevalence of vitamin B12 deficiency and its associated factors among patients with type 2 diabetes mellitus (DM) who are on metformin.

Methodology. A total of 205 patients who fit eligibility criteria were included in the study. A questionnaire was completed, and blood was drawn to study vitamin B12 levels. Vitamin B12 deficiency was defined as serum B12 level of ≤300 pg/mL (221 pmol/L).

Results. The prevalence of vitamin B12 deficiency among metformin-treated patients with type 2 DM patients was 28.3% (n=58). The median vitamin B12 level was 419 (±257) pg/mL. The non-Malay population was at a higher risk for metformin-associated vitamin B12 deficiency [adjusted odds ratio (OR) 3.86, 95% CI: 1.836 to 8.104, p<0.001]. Duration of metformin use of more than five years showed increased risk for metformin-associated vitamin B12 deficiency (adjusted OR 2.06, 95% CI: 1.003 to 4.227, p=0.049).

Conclusion. Our study suggests that the prevalence of vitamin B12 deficiency among patients with type 2 diabetes mellitus on metformin in our population is substantial. This is more frequent among the non-Malay population and those who have been on metformin for more than five years.

Key words: Vitamin B12, metformin, deficiency, type 2 diabetes mellitus, type 2 DM

INTRODUCTION

Type 2 diabetes mellitus is a major non-communicable disease in Malaysia for which metformin is one of the most commonly prescribed first line medications. Multiple cross-sectional studies have reported a wide range in prevalence of biochemical vitamin B12 deficiency with metformin exposure, ranging from 5.8% to as high as 30%.1,6 Vitamin B12 deficiency associated with metformin use is thought to occur due to vitamin B12 malabsorption at the terminal ileum.5,7

Vitamin B12 deficiency is clinically important as it is a reversible cause of bone marrow failure and nerve damage.8 Neurological damage as a result of metformin-induced vitamin B12 deficiency can present as peripheral neuropathy and may be mistaken for diabetic neuropathy.8 Because vitamin B12 deficiency and its associated complications are treatable and potentially reversible, early detection and treatment are clinically important in patients with diabetes who are on metformin.8

The first large scale study among Asians designed to investigate the prevalence and risk factors associated with vitamin B12 deficiency was conducted among Koreans in 2014. It reported vitamin B12 deficiency in 9.5% of the patients who were on metformin.9 Interestingly, another study among the South African population demonstrated that subjects of black South African descent on metformin had a lower prevalence of B12 deficiency, suggesting that different ethnic origins may influence the prevalence of metformin-associated vitamin B12 deficiency.10 This study is the first of its kind that investigated the association between ethnicity and vitamin B12 deficiency among metformin-treated type 2 DM patients.

Duration of use and dose of metformin have also been shown to influence vitamin B12 levels. A meta-analysis of six randomized controlled trials showed a significant...
reduction in vitamin B12 levels induced by metformin and suggested that this may be dose dependent. In another large study published in the same year, Korean patients on higher doses (metformin >1 g daily) and with longer treatment duration (>4 years) were more likely to be deficient in vitamin B12. 3,9,15

Some studies have found lower serum levels of vitamin B12 in smokers, but the exact mechanism for this is still poorly understood. 12 It is thought that smokers generally have poor dietary intake. The second National Health and Nutrition Survey (NHANES II) found that smokers have a lower intake of most vitamins and were less likely to have consumed fruit, vegetables, vitamins and mineral supplements. Proton pump inhibitors (PPI) and histamine 2 receptor antagonists (H2RA) may lead to malabsorption of vitamin B12 due to inhibition of gastric acid secretion and reduced production of the intrinsic factor. 13 Excessive alcohol intake is also linked to vitamin B12 deficiency. This has been attributed to intestinal malabsorption due to altered binding of intrinsic factor and alcohol-induced ileal damage. 10,14

The primary objective of this study is to determine the prevalence of vitamin B12 deficiency among patients with type 2 DM who are on metformin in Malaysia. Our secondary objective is to determine the associated factors contributing to vitamin B12 deficiency in this cohort.

METHODOLOGY

Study Population
This was a cross-sectional prevalence study. A total of 252 patients with type 2 DM were screened from two study centers in the district of Kuantan, Pahang in Malaysia. Patients who turned up for their scheduled clinic appointment at the type 2 diabetes clinic in the two centers were seen screened and recruited during their routine clinic visit between September 2018 and February 2019. Patients aged 18 years old and above with a diagnosis of type 2 DM who were on metformin for at least 6 preceding months were screened. Participants were recruited based on eligibility and willingness to participate. Forty-six patients were excluded based on the exclusion criteria, while one declined to join. Patients who had pernicious anaemia; prior bariatric surgery; gastrectomy, colectomy or inflammatory bowel disease; ongoing critical illnesses; malignancy; liver cirrhosis or renal impairment (creatinine ≥265 µmol/L) were excluded. Subjects who were vegetarians, recipients of vitamin B12 injections or supplements within the past 3 months, pregnant or lactating were excluded as well. Once informed consent was obtained, all participants were interviewed based on a standardized questionnaire (Appendix 1). Blood extraction for serum vitamin B12 levels was done. Vitamin B12 deficiency was defined as serum B12 level ≤300 pg/mL (221 pmol/L). This encompasses vitamin B12 levels defined as low and borderline low. 3,9,15 Serum vitamin B12 level was measured by chemiluminescent microparticle Intrinsic Factor assay using the 7K61 ARCHITECT B12 Reagent Kit. Sample size was calculated based on the 9.5% prevalence of B12 deficiency among patients with type 2 diabetes patients on metformin. 9 Using the sample size calculator for estimations with type I error probability and precision of 0.05, the required sample size was 178. 16 Sample size was augmented by 15% to take into account missing data. The final sample size was determined to be 205.

Statistical Analysis
Descriptive analyses of all the demographic and outcome variables were performed. Results of the continuous variables are described with mean and standard deviation or median and interquartile range and results of categorical variables are described with frequency and percentage. Test of normality was used to determine the distribution of the outcome variables. Independent sample t-test was used for normally distributed variables, and Mann-Whitney U-test or Fisher Exact test for variables with a skewed distribution. Pearson Chi-Square test was used to determine association between categorical predictors variables and outcome variables. The variables with p-value <0.2 in the univariate analysis were included in the multivariate analysis. Multiple logistic regression analysis was performed to assess the independent predictive effect of the variables on the risk for vitamin B12 deficiency. All statistical analyses were performed using Statistical Package for Social Science (SPSS) Version 22.0. A p-value of less than 0.05 was considered significant.

RESULTS
Two hundred fifty-two patients with type 2 DM were screened from two study centers. Forty-six patients were subsequently excluded. A total of 205 patients from two study centers were finally included in the study (Figure 1). Majority (51.7%, n=106) were recruited from a tertiary hospital while 48.3% (n=99) were from a health clinic.

Table 1 shows the baseline demographic data of our study population. A total of 79 (38.5%) males and 126 (61.5%) females were enrolled. Majority of the patients were of Malay race (78%) while the remaining were non-Malay (15.6% Chinese and 6.3% Indian). The median age of the

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**Figure 1.** Study design summarizing sample recruitment.
Vitamin B12 deficiency has been long known to adversely affect health, causing anaemia and neuropathy among other complications. Metformin, a widely used anti-diabetes drug, has been reported as a risk factor for vitamin B12 deficiency (OR 2.27, 95% CI: 1.21 to 4.27, p<0.01). The other studied factors did not reveal a significant association with vitamin B12 deficiency in our study population (Table 2).

In the multivariate analysis, after adjusting for age, smoking status, duration of diabetes and HbA1c, the non-Malay population remained at a significantly higher risk for metformin-associated vitamin B12 deficiency (adjusted OR 3.86, 95% CI: 1.86 to 8.104, p<0.001) (Table 3). Metformin use for a duration of more than five years showed an increased risk for metformin-associated vitamin B12 deficiency (adjusted OR 2.06, 95% CI: 1.003 to 4.227, p=0.049).

**DISCUSSION**

Vitamin B12 deficiency has been long known to adversely affect health, causing anaemia and neuropathy among other complications. Metformin, a widely used anti-diabetes drug, has been reported as a risk factor for vitamin B12 deficiency. To the best of our knowledge, this is the first study in Southeast Asia designed to investigate the prevalence vitamin B12 deficiency among metformin-treated patients with type 2 diabetes mellitus.
The prevalence of vitamin B12 deficiency in our study population is 28.3%, which falls at the upper end of global prevalence. The worldwide prevalence of vitamin B12 deficiency among metformin users ranges between 4.3 to 30%. Vitamin B12 deficiency associated with metformin use is thought to occur due to vitamin B12 malabsorption. It is postulated that metformin interferes with the calcium-dependent membrane action responsible for vitamin B12-intrinsic factor absorption in the terminal ileum. The substantial prevalence of vitamin B12 deficiency in our population should prompt consideration for routine screening of this deficiency among metformin-treated type 2 DM patients.

Our study demonstrated that race and duration of metformin use were the most consistent associated factors with vitamin B12 deficiency among metformin users. This association remained evident after adjusting for potential confounding factors by multivariate analysis. The most significant association was race. Non-Malay race was associated with an approximately four-fold increased risk for metformin-associated vitamin B12 deficiency even after adjusting for potential confounders (p<0.001). A study conducted in Africa found that Black South African descent was a significant protective factor for vitamin B12 deficiency among metformin-treated patients. This was the first study to report ethnic differences in vitamin B12 levels among metformin-exposed type 2 DM patients. Higher levels of the vitamin binding proteins transcobalamin II and haptocorrin in black individuals have been described in South African settings, explaining their relatively elevated vitamin B12 levels. The difference in prevalence of vitamin B12 deficiency among different ethnic groups in Asia has not been studied. The currently utilized cut-off points and definitions of vitamin B12 deficiency do not consider the possible effects of ethnicity. Further research is needed to determine why Malay ethnicity seemed protective against metformin-associated vitamin B12 deficiency.

Duration of metformin use of more than five years conferred a greater than two-fold increased risk for vitamin B12 deficiency (p=0.049) in our population. Several studies have shown a significant positive association between duration of metformin use and vitamin B12 deficiency. In a large-scale study among Koreans (n=799), daily metformin dosage and treatment duration were the most consistent risk factors for vitamin B12 deficiency. Secondary analysis from the Diabetes Prevention Program Outcomes Study (DPP-OS) showed that 13 years after randomization, there was a 13% increased risk for vitamin B12 deficiency per year of total metformin use. The results of our study echo these findings of increased risk for vitamin B12 deficiency with longer duration of metformin use.

Age, sex, body mass index, smoking, duration of diabetes and HbA1c levels did not show a statistically significant association with vitamin B12 deficiency in our population. There was no significant association between vitamin B12 deficiency and the use of other anti-diabetes medications (Table 2).

Previous studies have linked vitamin B12 deficiency with the use of PPI and HRA among metformin-treated patients. These observations were supported by the concept that gastric acidity is vital for vitamin B12 absorption, and that PPI and HRA result in reduction in acid discharge by gastric parietal cells. However, this finding was controversial. Our study did not find a significant association between use of PPI or HRA and vitamin B12 deficiency. This could be attributed to the very small number of patients in our study who were on PPI or HRA (n=5).

Vitamin B12 deficiency is clinically important as it can cause anemia, bone marrow failure, peripheral neuropathy and cognitive impairment. Neuropathy secondary to metformin-associated vitamin B12 deficiency may be mistaken for peripheral neuropathy secondary to diabetes-associated microvascular complications, as

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**Table 2. Univariate logistic regression analysis**

| Gender | OR* | 95% CI | p-value |
|--------|-----|--------|---------|
| Male   | 1.18 | 0.63 - 2.20 | 0.60 |
| Female | 1   |         |         |

| Race    | OR* | 95% CI | p-value |
|---------|-----|--------|---------|
| Non-Malay | 4.81 | 2.39 - 9.70 | <0.01 |
| Malay   | 1   |         |         |

| Daily metformin dose (g/day) | OR* | 95% CI | p-value |
|-----------------------------|-----|--------|---------|
| >1000 mg                    | 1.57 | 0.64 - 3.84 | 0.33 |
| ≤1000 mg                    | 1   |         |         |

| Metformin treatment duration | OR* | 95% CI | p-value |
|-----------------------------|-----|--------|---------|
| >5 years                    | 2.27 | 1.21 - 4.27 | 0.01 |
| ≤5 years                    | 1   |         |         |

| HbA1c ≤7% | OR* | 95% CI | p-value |
|-----------|-----|--------|---------|
| ≤7%       | 2.07 | 0.94 - 4.56 | 0.07 |
| >7%       | 1   |         |         |

| Age, yr | OR* | 1.03 | 1.00 - 1.06 | 0.06 |
|---------|-----|-------|-------------|------|
| BMI, kg/m² | 1.02 | 0.98 - 1.07 | 0.33 |
| Diabetes duration, month | 1.00 | 1.00 - 1.01 | 0.03 |
| Smoking | 2.45 | 0.90 - 6.70 | 0.08 |
| Sulfonylurea | 1.01 | 0.55 - 1.87 | 0.97 |
| DPP-4 inhibitor | 0.83 | 0.26 - 2.70 | 0.76 |
| SGLT2 inhibitor | 1.27 | 0.11 - 14.30 | 0.85 |
| PPI | 2.56 | 0.16 - 41.65 | 0.51 |
| HRA | 1.27 | 0.11 - 14.30 | 0.85 |

*OR, odds ratio
†HbA1c, glycosylated hemoglobin
‡BMI, body mass index
§DPP-4, dipeptidyl peptidase-4
¶SGLT2, sodium glucose cotransporter-2
∥PPI, proton pump inhibitor
††HRA, H receptor antagonist
**Table 3. Multiple logistic regression analysis**

| Non-Malay race | Adjusted OR* | 95% CI | P | Cov and Snell R square | Nagelkerke R square |
|----------------|--------------|--------|---|------------------------|-------------------|
|                 | 3.86 | 1.836 - 8.104 | <0.001 | 0.13 | 0.19 |

| Duration of metformin use >5 years | Adjusted OR* | 95% CI | P |
|-----------------------------------|--------------|--------|---|
| Non-smoker                        | 2.06 | 1.003 - 4.227 | 0.049 |
| Duration ≥7 years                 | 0.36 | 0.120 - 1.059 | 0.063 |
| HbA1c ≤7%                         | 2.32 | 0.934 - 5.751 | 0.070 |
| Constant                          | 3.24 | 0.056 | |

*OR, odds ratio
†HbA1c, glycosylated hemoglobin
both diseases can result in reduced vibration sense and diminished proprioception.\textsuperscript{8,26} There is no definitive clinical or electrophysiological test that can differentiate diabetic peripheral neuropathy from vitamin B12-associated neuropathy.\textsuperscript{3} This may lead to inappropriate use of tricyclic antidepressants and anticonvulsants to manage symptoms.\textsuperscript{8,27,28}

Recognition of metformin-associated vitamin B12 deficiency is imperative as it is potentially treatable and reversible. Multivitamin use seemed to protect type 2 DM patients from B12 deficiency.\textsuperscript{19} Randomized trials among adults taking supplemental vitamin B12 doses as low as 6 to 9 mcg daily show higher serum B12 levels compared with placebo.\textsuperscript{20,26}

We regard the prevalence of vitamin B12 deficiency of 28.3\% in our study population as a substantial percentage, as it affects over a quarter of the type 2 DM patients who are on metformin. This provides a valuable manual for clinicians to consider testing for vitamin B12 levels especially among type 2 DM patients who are of non-Malay race and who have been on metformin for more than five years. Although the clinical implication of our findings has not been demonstrated in this study, the potential complications of vitamin B12 deficiency has been well documented in literature.

Our study had several limitations. It was conducted in two centers from a single district, which may not be representative of the entire Malaysian population. We were unable to measure serum homocysteine and serum methylmalonic acid, early markers of vitamin B12 deficiency, as this was cost-prohibitive. The study was not sufficiently powered for some of the factors that were evaluated as an association with B12 deficiency in our population. We did not include a detailed dietary history which could be a potential contributing factor to low vitamin B12 levels.

**CONCLUSION**

Given the mounting evidence associating metformin exposure with low vitamin B12 levels, assessment of serum vitamin B12 levels among metformin-treated patients should be incorporated into routine clinical practice. According to the 2019 National Health and Morbidity Survey (NHMS), the prevalence of type 2 DM in Malaysia is 18.3\% among adults above the age of 18.\textsuperscript{18} Metformin is widely recommended as a first line agent in the treatment of type 2 DM patients in Malaysia. The prevalence of vitamin B12 deficiency in patients with type 2 DM on metformin is 28.3\% in our study population as a substantial percentage.

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**Statement of Authorship**

All authors certified fulfillment of ICMJE authorship criteria.

**Author Disclosure**

The authors declared no conflicts of interest.

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Appendix 1. Association of Vitamin B12 deficiency and Metformin in Type 2 Diabetes Mellitus

Data Collection Sheet

| Date | Centre |
|------|--------|
|      |        |

| Name of Investigator |
|----------------------|
|                      |

Subject Information

| Subject Initials | Subject ID (IC number) |
|------------------|------------------------|
|                  |                        |

| Gender | Race     |
|--------|----------|
| M/F    | Malay / Chinese / Indian / Others |

| Age (years) | Weight (kg) | BMI (kg/m²) |
|-------------|-------------|-------------|
|             |             |             |

| Current Smoker | Alcohol consumption (units/week or units/month- see appendix) |
|----------------|--------------------------|
| Yes / No       |                          |

| Duration of diabetes mellitus (since diagnosis) | Current metformin dose (daily dose) |
|-------------------------------------------------|------------------------------------|
|                                                 |                                    |

| Total duration of metformin use (in years/months) |
|--------------------------------------------------|
|                     |

| Medications: (tick relevant box) |
|---------------------------------|
| Sulphonylureas                  |
| Proton pump inhibitor (PPI)     |
| DPP-IV antagonist               |
| H₂ antagonist                   |
| GLP-1 agonist                   |
| Statin                          |
| SGLT-2 inhibitor                |
| AACE/IRB                        |
| Alpha glucosidase inhibitor     |
| Aspirin                         |
| Thiouolidinediones              |
| Insulin                         |

| Others (please specify) |
|-------------------------|
|                         |

Lab Investigations

| Test                                | Value | Date |
|-------------------------------------|-------|------|
| Vitamin B12 level (pg/mL)           |       |      |
| Fasting Blood Sugar (mmol/L)        |       |      |
| HbA1c (%) (last available value)    |       |      |

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