

# Metformin-associated vitamin B12 deficiency in the elderly

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## ABSTRACT

**Objective.** To compare the prevalence of the vitamin B12 deficiency in geriatric diabetic patients treated with or without metformin.

**Methods.** Records of 134 patients with diabetes mellitus (DM) aged 61 to 93 (mean, 80) years who were treated with (n=56) or without (n=78) metformin were reviewed. Patient demographics (age, gender, duration of DM, smoking status, alcohol consumption), medication parameters (daily dosage and duration of metformin therapy), and haematological parameters (haemoglobin level, mean corpuscular volume [both of which may reflect vitamin B12 deficiency], serum vitamin B12, and folate level) were recorded. Definite deficiency was defined as serum vitamin B12 level of <150 pmol/L, whereas possible deficiency as <220 pmol/L.

**Results.** The mean serum vitamin B12 level was lower in the metformin group (282.1 vs. 380.1 pmol/L,  $p=0.023$ ). 15% and 37% of these patients had definite and possible vitamin B12 deficiency, respectively. The metformin group had significantly higher prevalence of definite deficiency (29% vs. 5%,  $p<0.001$ ) and possible deficiency (52% vs. 27%,  $p<0.03$ ). Odds ratios of definite and possible deficiency in the metformin group were 7.40 (95% CI, 2.32-23.62;  $p=0.001$ ) and 2.92 (95% CI, 1.41-6.02;  $p=0.004$ ), respectively. Within the metformin group, the mean serum vitamin B12 level was significantly lower in those on high dosage (173 vs. 315 pmol/L,  $p<0.005$ ).

**Conclusion.** Metformin use was significantly associated with vitamin B12 deficiency. Physician should check the patient's baseline vitamin B12 level, and serially monitor vitamin B12 levels and nutritional status of those treated with metformin, and prescribe vitamin B12 supplement if necessary.

**Key words:** Diabetes mellitus, type 2; Metformin; Vitamin B 12 deficiency

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## INTRODUCTION

Metformin may be associated with vitamin B12 deficiency in elderly patients with diabetes mellitus (DM),<sup>1</sup> which is a common disease among older people. Almost 50% of type-2 diabetic patients are over 60 years old.<sup>2</sup> In the United States, more than 10 million persons over the age of 60 years have DM. This amounts to a prevalence of about 21% in this age group as compared to about 10% in younger

adults. Annually, approximately 575 000 people aged  $\geq 60$  years are diagnosed as having diabetes.<sup>3</sup> According to the World Health Organization, the number of type-2 DM patients is expected to double within the next 25 years. In Hong Kong, diabetes is the ninth commonest cause of death and accounts for 1.6% of all deaths in 2005.<sup>4</sup> In a population-based survey in Hong Kong in 1995, the age-adjusted prevalence of type-2 diabetes was 8.5%. The crude prevalence ranged from 1.7% in persons aged 25 to

34 years, compared to >25% in those older than 65 years.<sup>5</sup> Older adults with diabetes are at higher risk of incontinence,<sup>6</sup> falls,<sup>7</sup> cognitive impairment,<sup>8</sup> and depressive symptoms.<sup>9</sup> DM is also a predictor for functional decline in older adults and is associated with increased difficulty in activities of daily living (ADL) and instrumental ADL.<sup>10</sup>

The United Kingdom Prospective Diabetes Study demonstrated that the biguanide metformin is as effective as sulfonylurea for glycaemic control and has additional cardiovascular protective effects.<sup>11</sup> The American Diabetes Association suggests using metformin as the first-line medical therapy for type-2 diabetes.<sup>12</sup> When used alone, metformin rarely causes hypoglycaemia in older people. It increases insulin sensitivity and improves weight loss and the lipid profile. Its side effects include lactic acidosis in patients who experience heart failure, renal failure, and among alcoholic patients, as well as vitamin B12 deficiency. 30% of patients have vitamin B12 malabsorption after biguanide treatment, although it is uncertain whether this is due to DM itself or to biguanides.<sup>1</sup>

Metformin does not alter intestinal motility or cause bacterial overgrowth in the gut.<sup>13</sup> Biguanide interacts with a complex of intrinsic-factor/vitamin B12 and cubilin, which is an endocytic receptor involved in the absorption. The vitamin B12-intrinsic factor complex is taken up by the ileal cell surface by a calcium-dependent process, which is affected by metformin via impaired calcium availability.<sup>14,15</sup> The hydrophobic tail of biguanides such as metformin, extends into the hydrocarbon core of membranes. The protonated biguanide group gives a positive charge to the surface of the membrane, which displaces divalent cations.<sup>16</sup> Thus, the biguanides alter membrane potentials and affect their calcium-dependent functions. Metformin also has an effect on the cubilin, which may affect B12-intrinsic factor complex absorption and result in the deficiency.

## METHODOLOGY

This observational, cross-sectional study compared the prevalence of vitamin B12 deficiency in 134 DM patients aged 61 to 93 (mean, 80) years who were treated with (n=56) or without (n=78) metformin from September 2005 to June 2006. Patients were excluded if they had other conditions associated with vitamin B12 deficiency, including vegetarian

diet (n=7), pernicious anaemia (positive schilling test result or positive of anti-intrinsic factor antibodies, n=10), pancreatic exocrine insufficiency, and/or a history of gastrectomy or small bowel resection (n=7). Patients were also excluded if in the previous 3 months they had received oral or intramuscular vitamin B12 supplementation or non-oral feeding that potentially contained vitamin B12 (n=5). Owing to the long half life of vitamin B12 in body stores, the 3-month exclusion period was selected, thus ensuring an adequate washout period.

Patient demographics (age, gender, duration of DM, smoking status, alcohol consumption), medication parameters (daily dosage and duration of metformin therapy), and haematological parameters (haemoglobin level, mean corpuscular volume [both of which may reflect vitamin B12 deficiency], serum vitamin B12, and folate level) were recorded. Two reference levels for the vitamin B12 deficiency were used: definite deficiency (<150 pmol/L) and possible deficiency (<220 pmol/L).<sup>17-22</sup>

Diabetic older people were routinely screened for diabetic control (HbA1c and spot blood glucose) and nephropathy (renal function test). Vitamin B12, folate HbA1c, and renal function testing were also requested.

The prevalence of vitamin B12 deficiency was calculated for the metformin and non-metformin groups. Potential factors associated with vitamin B12 deficiency were examined using the Chi squared test or Fisher's Exact test (for categorical variables) or independent sample *t* tests (for continuous variables). The binary logistic regression model was used for both univariate and multivariate analyses to calculate the odds ratios (OR). The 95% confidence intervals (CI) were based on likelihood. All *p* values were 2-sided and considered significant if <0.05.

## RESULTS

The mean patient ages of the metformin and non-metformin groups were 79.3 and 80.5 years, respectively. The 2 groups were not significantly different in terms of age, gender, smoking status, alcohol consumption, and serum levels of folate and HbA1C (TABLE 1).

The mean serum vitamin B12 level was lower

in the metformin group (282.1 vs. 380.1 pmol/L,  $p=0.023$ , **TABLE 1**). 15% and 37% of these patients had definite and possible vitamin B12 deficiency, respectively (**TABLE 2**). The metformin group had significantly higher prevalence of definite deficiency (29% vs. 5%,  $p<0.001$ ) and possible deficiency (52% vs. 27%,  $p<0.03$ ) [**TABLE 2**]. Odds ratios of definite and possible deficiency in the metformin group were 7.40 (95% CI, 2.32-23.62;  $p=0.001$ ) and 2.92 (95% CI, 1.41-6.02;  $p=0.004$ ), respectively (**TABLE 3**). Eight

patients in the metformin group and 3 in the non-metformin group had severe vitamin B12 deficiency ( $<100$  pmol/L).

Within the metformin group, the mean serum vitamin B12 level was significantly lower in those on high dosage ( $>1500$  mg per day) [173 vs. 315 pmol/L,  $p<0.005$ , **FIGURE**], but not significantly different between long-term ( $>3$  years) and short-term users ( $p=0.546$ ).

**TABLE 1**  
Patient characteristics of metformin and non-metformin groups\*

Characteristics	Metformin group (n=56)	Non-metformin group (n=78)	p Value
Age (years)	79.7±5.5	80.5±6.7	0.782
Male	35 (45)	20 (36)	0.288
Vitamin B12 (pmol/L)	282.1±245.6	381.0±245.9	0.023
Folate (ng/mL)	24.7±10.0	23.6±10.3	0.544
Haemoglobin (g/dL)	11.7±1.44	11.8±1.70	0.836
Glycylated haemoglobin (%)	6.8±1.11	7.00±1.2	0.673
Mean corpuscular volume (fL)	86.3±12.54	87.7±7.16	0.438
Albumin (g/L)	36.2±7.0	34.1±5.63	0.059
Smoker	22 (28)	20 (36)	0.355
Alcohol use	18 (23)	9 (16)	0.319
H2 blocker/ proton pump inhibitor use	10 (13)	4 (7)	0.289

\* Data are presented as mean±SD or No. (%) of patients

**TABLE 2**  
Prevalence of definite and possible vitamin B12 deficiency in metformin and non-metformin groups

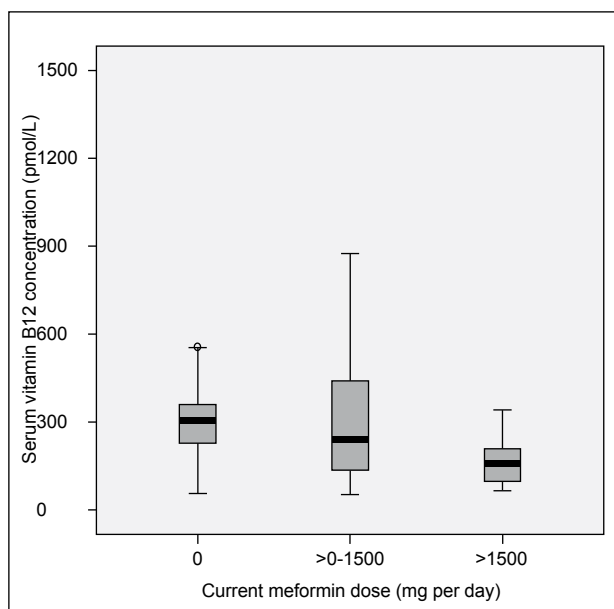
Group	No. (%) of patients	
	Definite deficiency ( $<150$ pmol/L)	Possible deficiency ( $<220$ pmol/L)
Metformin (n=56)	16 (29)*	29 (52) <sup>†</sup>
Non-metformin (n=78)	4 (5)	21 (27)
Total	20 (15)	50 (37)

\*  $p<0.05$

<sup>†</sup>  $p<0.001$

**TABLE 3**  
Risk factors of vitamin B12 deficiency

Risk factor	Adjusted OR (95% CI)			
	Definite deficiency ( $<150$ pmol/L)	p Value	Possible deficiency ( $<220$ pmol/L)	p Value
Age	1.01 (0.91-1.13)	0.83	1.00 (0.91-1.10)	0.99
Serum folate	1.03 (0.97-1.10)	0.27	1.02 (0.97-1.08)	0.41
Glycylated haemoglobin	1.58 (0.92-2.70)	0.10	0.71 (0.43-1.17)	0.18
Metformin (all)	7.40 (2.32-23.62)	0.001	2.92 (1.41-6.02)	0.004
Metformin (high) [ $\geq 1.5$ g/day]	1.15 (0.30-4.44)	0.84	2.59 (0.69-9.70)	0.159



**FIGURE. Serum vitamin B12 concentrations in patients with different dosages of metformin: the lower and the upper bounds of the boxes represent the 25th and 75th percentiles, respectively. The horizontal lines in the boxes correspond to the median values. The lower and upper error bars indicate the 10th and 90th percentiles, respectively.**

## DISCUSSION

Metformin has some effects on reducing the level of serum vitamin B12 or its metabolites. Metformin is involved in 14 to 22% of patients with definite vitamin B12 deficiency.<sup>23,24</sup> Metformin may result in cobalamin malabsorption secondary to the impaired release of vitamin B12 from food or its binding protein. The protonated biguanide group gives a positive charge to the surface of the membrane, which acts to displace divalent cations.<sup>16</sup> Thus, metformin alters membrane potentials and affects divalent membrane cations. A higher dosage of metformin might lead to stronger or higher positive charge on the surface of the cell membrane by the protonated biguanide group. It affects the cubilin function and results in impaired vitamin B12 absorption. A daily dose of 1500 mg metformin was considered high in this study, because the clinically significant response to metformin is generally not seen at a daily dose of <1500 mg.<sup>25</sup> The serum vitamin B12 level should be monitored for patients receiving high daily dosage, and dosage should be reduced when patients develop vitamin B12 deficiency. Further studies are required to validate whether dosage reduction improves the deficiency.

We used 3 years as the cut-off point because most of our patients had been treated with metformin for a long period. Thus, we could not evaluate the short-term effect of metformin use on vitamin B12 deficiency. 16 weeks of treatment with metformin is associated with a significant decrease in vitamin B12 in 14% of the subjects.<sup>24</sup> Serial monitoring of the vitamin B12 level is required, in particular at the start of metformin treatment. Baseline vitamin B12 level should also be checked prior commencing metformin treatment.

There is no consensus on the cut-off point of vitamin B12 deficiency. We defined definite and possible deficiency as serum vitamin B12 levels of <150 and <220 pmol/L, respectively.<sup>17-21</sup> Nonetheless, patients with varying clinical features of vitamin B12 deficiency may be regarded as within normal range.<sup>17,26</sup> In adults, a vitamin B12 level of 150 pmol/L is considered the lowest level for an adequate state. In a developing deficiency, serum concentrations are maintained by depleting body storage. Therefore, a concentration of 150 pmol/L might not reflect a sufficient vitamin B12 status,<sup>27</sup> and a cut-off value of <220 pmol/L is proposed.<sup>21</sup> Patients with subtle deficiency (150 to 220 pmol/L) may benefit from vitamin B12 supplement.

Polypharmacy may also affect the vitamin B12 level. Medications that may decrease serum vitamin B12 level include acid-suppressive therapy, i.e. histamine-2 receptor antagonist (H-2 blockers) or proton pump inhibitor.<sup>28</sup> We intended to examine the effect of polypharmacy on the vitamin B12 deficiency and to determine whether the acid-suppressive therapy was a confounder of serum vitamin B12 level. However, only a small proportion of our patients were receiving such therapy, and the association between polypharmacy and vitamin B12 deficiency could not be established. Physicians should be aware of the problem of polypharmacy or iatrogenesis in geriatric patients.

Anaemia and excessive mean corpuscular volume (MCV) are well-known clinical parameters indicative of vitamin B12 deficiency. A case of megaloblastic anaemia secondary to vitamin B12 malabsorption and long-term metformin treatment has been reported.<sup>29</sup> However, there was no significant difference between our metformin and non-metformin groups in terms of the haemoglobin

level and MCV. Indeed, haematological indices are not a reliable guide for diagnosing subtle vitamin B12 deficiency.<sup>30,31</sup> Anaemia tends to occur only when metabolic deficiency is moderately severe<sup>32</sup> or the deficiency is severe enough to affect the haematological indices. In addition, macrocytosis can be masked by coexisting microcytic processes including thalassaemia and iron deficiency.<sup>33</sup>

### Limitation

Patients attending the geriatric clinic were relatively older than the general population, resulting in possible selection bias. Metformin-related vitamin B12 deficiency in younger age-groups needs further studies.

We used serum vitamin B12 levels only to define deficiency. Metabolites (such as methylmalonic acid and total homocysteine) were not measured. They are considered more sensitive indicators of vitamin B12 status than plasma vitamin B12 levels, which have limited specificity and controversial sensitivity.<sup>34</sup> However, the assay for methylmalonic acid is complex, costly, and slow processing, whereas total homocysteine increases in patients with folate deficiency.

The amount of the vitamin B12 intake was not recorded. Daily intake of the vitamin B12 can be estimated from food recall records, which should have been carried out prior to blood sample taking. Details of food intake such as beef, pork, chicken, fish or egg, and milk products should have been recorded to estimate the amount of daily vitamin B12 intake.

### CONCLUSION

Metformin use was significantly associated with vitamin B12 deficiency. Subjects taking higher dosage tended to have lower serum vitamin B12 levels. Physician should check the patient's baseline vitamin B12 level, and serially monitor vitamin B12 levels and nutritional status of those treated with metformin, and prescribe vitamin B12 supplement if necessary.

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