Risk Factors of Vitamin B12 Deficiency in Patients Receiving Metformin

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Background: Identification of risk factors for metformin-related vitamin B12 deficiency has major potential implications regarding the management of diabetes mellitus.

Methods: We conducted a nested case-control study from a database in which the source population consisted of subjects who had levels of both serum vitamin B12 and hemoglobin A1c checked in a central laboratory. We identified 155 cases of diabetes mellitus and vitamin B12 deficiency secondary to metformin treatment. Another 310 controls were selected from the cohort who did not have vitamin B12 deficiency while taking metformin.

Results: A total of 155 patients with metformin-related vitamin B12 deficiency (mean ± SD serum vitamin B12 concentration, 148.6 ± 40.4 pg/mL [110 ± 30 pmol/L]) were compared with 310 matched controls (466.1 ± 330.4 pg/mL [344 ± 244 pmol/L]). After adjusting for confounders, we found clinically important and statistically significant association of vitamin B12 deficiency with dose and duration of metformin use. Each 1-g/d metformin dose increment conferred an odds ratio of 2.88 (95% confidence interval, 2.15-3.87) for developing vitamin B12 deficiency (P < .001). Among those using metformin for 3 years or more, the adjusted odds ratio was 2.39 (95% confidence interval, 1.46-3.91) (P = .001) compared with those receiving metformin for less than 3 years. After exclusion of 113 subjects with borderline vitamin B12 concentration, dose of metformin remained the strongest independent predictor of vitamin B12 deficiency.

Conclusions: Our results indicate an increased risk of vitamin B12 deficiency associated with current dose and duration of metformin use despite adjustment for many potential confounders. The risk factors identified have implications for planning screening or prevention strategies in metformin-treated patients.

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Metformin has greatly improved the prognosis of diabetic patients by improving insulin sensitivity and protection against vascular complications. The United Kingdom Prospective Diabetes Study (UKPDS) confirmed the long-term benefit of metformin in decreasing diabetes-related end points, diabetes-related death, and all-cause mortality in overweight patients with diabetes mellitus, and with less weight gain and fewer hypoglycemic attacks than occurred with insulin and sulphonylureas treatment.

Evidence from early clinical observation, however, indicated a prevalence of 30% for vitamin B12 malabsorption among patients undergoing long-term metformin treatment. Subsequent studies reported that metformin decreased serum vitamin B12 level by 14% to 30%. The findings of metformin-related vitamin B12 deficiency have caused us to question whether this adverse effect is predictable among patients with type 2 diabetes mellitus who receive metformin. To date, knowledge of the risk factors of this adverse event of metformin is still limited. From a clinical standpoint, characterization of risk factors for metformin-related vitamin B12 deficiency is the key to better patient care. First, there is likely to be an improved yield of detecting vitamin B12 deficiency if high-risk individuals can be identified. Second, subjects identified as having substantial risk for metformin-related vitamin B12 deficiency might benefit from empirical screening or primary prevention with other means such as calcium supplementation.

METHODS

We undertook a nested case-control study in the New Territories East Cluster region, Hong Kong, between January 2003 and November 2005. A
database was generated by a central chemical pathology laboratory that provides vitamin B$_{12}$ assay service to cover 19% (1.3 million) of the population in Hong Kong. In other words, we nested the case-control study within a source cohort population, as defined by patients who had levels of both hemoglobin A$_{1c}$ and serum vitamin B$_{12}$ being checked for clinical reasons within the study period. To ensure complete data extraction, we also restricted the subjects to have at least 1 year of continuous medical history recorded on computer. Serum vitamin B$_{12}$ and folate concentrations were determined by electrochemiluminescent immunoassay (E170 Analytic; Roche Diagnostics, Indianapolis, Ind). The mean±SE lower limit of normal by our vitamin B$_{12}$ assay was 253.4±50.1 pg/mL (187±37 pmol/L); therefore, we chose a cutoff point of 203.3 pg/mL (150 pmol/L) for vitamin B$_{12}$ deficiency. Serum methylmalonic acid concentration evaluation was not routinely performed.

We defined a case as a Chinese subject with diabetes mellitus and vitamin B$_{12}$ deficiency during metformin treatment after excluding subjects who had pernicious anemia (positive Schilling test result or anti-intrinsic factor antibodies), pancreatic exocrine insufficiency, and/or history of gastrectomy or small bowel resection. To avoid potentially confounding causes of vitamin B$_{12}$, patients receiving oral or parenteral vitamin B$_{12}$ supplementation within 3 months prior to study initiation were excluded. Two controls were selected for each case, matched according to the date of blood sampling. Eligible controls for each case were individuals within our database with diabetes mellitus who did not have vitamin B$_{12}$ deficiency (serum concentration >203.3 pg/mL [150 pmol/L]) while taking metformin.

Standardized data collection forms were used to abstract information from computerized medical records and pharmacy records. Data collected included demographic information, concentrations of serum vitamin B$_{12}$, folate, and blood hemoglobin, white blood cell and platelet counts, mean corpuscular volume, documentation of concomitant histamine H$_{3}$ receptor antagonist or proton pump inhibitor therapy, vegetarian diet, smoking history, and alcohol consumption. Detailed data of metformin daily dose and duration of metformin therapy at the time of blood collection were also collected.

Mean and standard deviation values for continuous variables were calculated to characterize our study population. The association between discrete variables and metformin-related vitamin B$_{12}$ deficiency was assessed by cross-tabulation and the chi$^2$ test or Fisher exact test, whereas continuous variables between groups were compared with unpaired t test or the nonparametric Wilcoxon rank sum test as appropriate. To estimate the strength of association, a binary logistic regression model was used for both univariate and multivariate analysis to calculate the odds ratio (OR) of metformin-related vitamin B$_{12}$ deficiency. Our model was constructed with stepwise selection for relevant variables by previous studies and those associated with metformin-related vitamin B$_{12}$ deficiency at an alpha level of <.1 in our univariate analysis. Because of the lack of a precise standard and uncertainty of definition of vitamin B$_{12}$ deficiency without supplementary methylmalonic acid measurement, we repeated the regression analysis after excluding cases with borderline vitamin B$_{12}$ concentration (>203.3 and ≤298.1 pg/mL [>150 and ≤220 pmol/L]) to minimize miscategorization error. Confidence intervals (CIs) reported are likelihood based. All P values were 2-sided, and we regarded P <.05 as significant. All statistical analyses were performed using The Statistical Package for the Social Sciences (Windows, version 13.0; SPSS Inc, Chicago, Ill).

**RESULTS**

Between January 2003 and November 2005, a total of 3987 source subjects were evaluated for the study. Figure 1 summarizes the trial profile. Of 355 cases with a laboratory finding of vitamin B$_{12}$ deficiency (serum vitamin B$_{12}$ concentration ≤203.3 pg/mL [≤150 pmol/L]), 155 cases fulfilled the case selection criteria. We therefore identified 155 cases (mean±SD serum vitamin B$_{12}$ concentration, 148.6±40.4 pg/mL [110±30 pmol/L]; range, 50.1-203.3 pg/mL [37-150 pmol/L]) and 310 matched controls (serum vitamin B$_{12}$ concentration, 466.1±330.4 pg/mL [344±244 pmol/L]; range, 204.6-2000.0 pg/mL [151-1476 pmol/L]) from our database.

Characteristics of the cases and controls are listed in Table 1. There were no significant differences in serum folate concentration between these 2 groups. Sex distribution, drinking, and smoking habits were similar among cases and controls. Vegetarians appeared to be more common among cases than controls, with borderline statistical significance (prevalence 2.6% vs 0.3%; P =.04). There was no substantial difference in age, with a mean±SD age of 72.5 ± 9.3 years among cases vs 71.4 ± 11.2 years among controls (P =.24).

In the univariate analysis, vegetarians (OR, 8.19; 95% CI, 0.91-73.9) had a borderline significant association with the risk of metformin-related vitamin B$_{12}$ deficiency. As summarized in Table 2, the unadjusted analysis showed a significant increase in risk associated with the current dose and duration of metformin medication. The metformin-treated patients with vitamin B$_{12}$ deficiency had a mean±SD daily dose of 2.0±0.7 g (interquartile range, 1.5-3.0 g), whereas the control group received a daily metformin dose of 1.4±0.7 g (interquartile range, 1.0-2.0 g). The crude OR for metformin-related vitamin B$_{12}$ deficiency was 2.37 (95% CI, 1.60-3.52) for a history of metformin use longer than 3 years. There was a significant increased risk of metformin-related vitamin B$_{12}$ deficiency associated with each additional 1-g/d dose increment of metformin (OR, 2.61; 95% CI, 2.00-3.42) (P <.001).

Once adjustments were made for potential confounding variables (Table 2), the most significant OR was as-

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**Figure 1.** Patient profile of the nested case-control study. Cases refer to subjects with diabetes mellitus and vitamin B$_{12}$ deficiency during metformin therapy, whereas the controls had metformin-treated diabetes mellitus but no vitamin B$_{12}$ deficiency. Cases and controls were matched according to the date of blood sampling. To convert vitamin B$_{12}$ to picograms per milliliter, divide by 0.738.
associated with current dose of metformin. This was followed by duration of metformin use and patient age. Each 1-g/d dose increment conferred a more than 2-fold increased risk of developing vitamin B12 deficiency with metformin (adjusted OR, 2.88; 95% CI, 2.15-3.87) (P < .001). Compared with metformin users of less than 3 years, the adjusted OR was 2.39 (95% CI, 1.46-3.52) (P <.001) for users of metformin for 3 years or more. Increased age appeared to be positively related to metformin-related vitamin B12 deficiency albeit with negligible clinical significance (adjusted OR, 1.36 for each 10-year increment in age). Vegetarian diets were also associated with vitamin B12 deficiency, but the confidence interval was wide (OR, 16.2; 95% CI, 1.69-154.00). We found no significantly increased risk for concurrent use of histamine H2 receptor antagonist or proton pump inhibitor.

We repeated the analyses after excluding 113 subjects (24%) with borderline vitamin B12 concentration between 203.3 and 298.1 pg/mL (150 and 220 pmol/L). This did not significantly influence the results (Table 3); current dose of metformin remained the strongest independent predictor of vitamin B12 deficiency (OR, 3.75 for each 1-g/d dose increment; 95% CI, 2.63-5.35) (P < .001). Of the 113 subjects excluded, their characteristics, including dose and duration of metformin use, were similar to the control subjects with serum vitamin B12 concentration exceeding 298.1 pg/mL (220 pmol/L) (details not shown).

We also investigated the effects of metformin dose on the serum vitamin B12 concentration among the cases. Cases were divided into 3 groups according to their current daily metformin doses: 1.0 g or less (n = 29), more than 1.0 g to 2.0 g (n = 76), or more than 2.0 g to 3.0 g (P < .001 by analysis of variance) (Figure 2). Post hoc analysis performed by the Bonferroni-adjusted pairwise comparisons revealed significantly lower vitamin B12 concentration in cases receiving more than 2.0 to 3.0 g/d than in those receiving more than 1.0 to 2.0 g/d (P = .01) and those receiving 1.0 g/d or less (P < .001). Conversely, when all the cases and controls in this study were included in the post hoc analysis, there was also a significant difference in the proportion of subjects with deficient vitamin B12 concentrations according to the daily metformin doses. The distribution of subjects (Figure 3) varied with the metformin doses with respect to the vitamin B12 concentration.
In our nested case-control study of metformin-related vitamin B12 deficiency, metformin dose and treatment duration emerged as the most consistent risk factors of vitamin B12 deficiency within a Chinese population with diabetes mellitus. Of special interest is that their association remained stable after adjustment for potential confounding factors in the multivariate analysis, thus reinforcing our conclusion that higher metformin dose and longer treatment duration are independent risk factors. There is also evidence from our post hoc analysis that serum vitamin B12 concentration showed a dose-dependent decrease with increasing dose of metformin.

It is impossible to deduce from our case-control study the mechanism of metformin-related vitamin B12 deficiency. The literature has reported that vitamin B12 deficiency may result from disorders in intestinal mobility and/or bacterial overgrowth. However, more recent evidence has demonstrated that metformin administration neither alters the intestinal motility nor causes bacterial overgrowth. On the other hand, metformin may disrupt the ileal vitamin B12 absorption. The vitamin B12-intrinsic factor complex is dependent on the luminal calcium concentration to facilitate uptake by the ileal cell surface receptor, whereas metformin is believed to give a positive charge to the surface of the membrane, which would act to displace divalent cations such as calcium. Impaired calcium availability due to metformin activity would therefore interfere with the calcium-dependent process of vitamin B12 absorption. It should be noted that our study design precluded a full assessment of the dietary or supplementary intake of calcium. Practically, our data and the graded relationship of metformin dose with serum vitamin B12 concentration (Figure 2) support the notion of a causal relation between metformin administration and vitamin B12 deficiency rather than suggesting any particular mechanism(s).

Unlike previous studies, our study demonstrated no excess risk of vitamin B12 deficiency among metformin-related vitamin B12 deficiency, Excluding Borderline Deficiency

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Crude OR (95% CI)</th>
<th>P Value</th>
<th>Adjusted OR (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (per 10-y increment)</td>
<td>1.21 (0.99-1.47)</td>
<td>.06</td>
<td>1.60 (1.24-2.04)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Vegetarian</td>
<td>5.19 (3.7-6.9)</td>
<td>.001</td>
<td>10.9 (10.9-109.0)</td>
<td>.04</td>
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<td>Use of histamine H2-receptor antagonist or proton pump inhibitor</td>
<td>8.34 (5.97-11.9)</td>
<td>.001</td>
<td>15.6 (12.0-20.0)</td>
<td>.001</td>
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<tr>
<td>Daily dose of metformin (per 1-g increment)</td>
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<td>.001</td>
<td>3.75 (2.63-5.35)</td>
<td>.001</td>
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<tr>
<td>Use of metformin for more than 3 y</td>
<td>2.98 (1.92-4.64)</td>
<td>.001</td>
<td>2.39 (1.46-3.91)</td>
<td>.001</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; OR, odds ratio.

Figure 2. Box-and-whisker plot shows the serum vitamin B12 concentration for cases of metformin-related vitamin B12 deficiency according to the different daily doses of metformin received. The lower and upper bounds of the boxes denote the 25th and 75th percentiles, respectively, and the horizontal lines in the boxes correspond to the median value. The lower and upper error bars indicate the 10th and 90th percentiles, respectively. To convert vitamin B12 to picograms per milliliter, divide by 0.738.

Figure 3. Effect of daily metformin dose on the proportion of all subjects with serum vitamin B12 concentration up to 203.3 pg/mL and up to 298.1 pg/mL. Error bars indicate standard error of the mean. To convert vitamin B12 to picograms per milliliter, divide by 0.738.

<220 pmol/L
≤150 pmol/L
min users who concurrently use H2-blockers or proton pump inhibitors. Theoretically, these medications can produce malabsorption of dietary protein-bound vitamin B12. The lack of association in the current study may stem from imprecise hospital-based medication records. We were unable to track the use of H2-blockers or proton pump inhibitors that are nowadays commonly prescribed by family practitioners or purchased over the counter. Such misclassification of exposure status (to H2-blockers or proton pump inhibitors), if present and nondifferential, would have had the effect of biasing the OR toward 1.

To minimize the risk of overmatching, no matching (other than the date of blood sampling) was used in this study, which might have hindered informative results or introduced confounding if the matching factor were linked with the risk of vitamin B12 deficiency. Subjects in our study, nevertheless, needed to have at least 1 serum vitamin B12 sample checked for a clinical purpose before being eligible as controls. Patients with certain characteristics may therefore have been more likely to be recruited as controls. Clinicians are in general more inclined to screen for vitamin B12 deficiency among vegetarians or the geriatric population, for instance, when a hematologic, neuropsychiatric, or cognitive disorder is either detected or suspected. If this had been the case, we would have expected the strength of association between metformin-related vitamin B12 deficiency and elderly or vegetarian subjects, if any, to be negated. This is a genuine possibility in the current study because statistically significant relationships between increased age, vegetarianism, and the development of vitamin B12 deficiency are apparent in the multivariate but not the univariate analysis.

Our results have to be interpreted with caution in light of several other limitations. One potential limitation of our study was the use of serum vitamin B12 concentration alone to define vitamin B12 deficiency, without metabolite (plasma homocysteine or methylmalonic acid) measurements for confirmation. Controversy exists as to whether serum measurement accurately reflects storage levels of vitamin B12. The possibility exists that bias may have been introduced by misclassifying cases and controls based on serum vitamin B12 measurement. Nonetheless, repeated analysis after our careful exclusion of subjects with borderline serum vitamin B12 data did not qualitatively affect the findings (Table 3), which suggests that any biases thus introduced were likely to be small. Furthermore, the retrospective nature of information retrieval for our subjects led to predictable limitations of data completeness. Another potential bias arises from the unblinded data acquisition method. Finally, the data from this study cannot be used to assess the incidence of vitamin B12 deficiency with metformin use.

In conclusion, this nested case-control study in a Chinese population showed that the risk of vitamin B12 deficiency is magnified in patients who have received both a higher dose and longer course of metformin treatment, independent of other clinical variables. Although this observational study may be subject to residual confounding that cannot be fully corrected for, we believe our findings should reinforce the heightened vigilance about vitamin B12 deficiency. Enough concerns exist to call attention to the value of vitamin B12 screening, particularly among at-risk patients receiving metformin. Our data underscore the need for monitoring subjects undergoing high-dose and/or prolonged-course metformin therapy.

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REFERENCES