The Prevalence of Vitamin B_{12} Deficiency in Patients with Type 2 Diabetes: A Cross-Sectional Study

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Purpose: **The purpose of this study is to define the prevalence of vitamin B_{12} deficiency in a type 2 diabetic population within a primary care practice. Metformin use and advanced age are associated with vitamin B_{12} deficiency and often present in type 2 diabetic patients, yet the prevalence of vitamin B_{12} deficiency in the diabetic population is unknown.**

Methods: **We conducted a cross-sectional study of 203 outpatient type 2 diabetic patients at a large military primary care clinic. Patients completed a survey and had B_{12} levels measured. Patients with borderline B_{12} levels also had methylmalonic acid and homocysteine levels drawn. Serum B_{12} levels <100 pg/mL or serum B_{12} levels of 100 to 350 pg/mL with elevation of serum methylmalonic acid >243 nmol/L or homocysteine >11.9 nmol/L defined B_{12} deficiency. Descriptive statistics described frequency and means. χ^2 and student's *t* tests were used to analyze associations between categorical and continuous variables, respectively. Multivariate logistical regression identified covariates independently associated with B_{12} deficiency.**

Results: **Twenty-two percent (n = 44) of diabetic patients had metabolically confirmed B_{12} deficiency. Patients on metformin had lower serum B_{12} levels (425.99 pg/mL vs 527.49 pg/mL; $P = .012$) and were at increased risk for B_{12} deficiency ($P = .04$), as defined by a serum B_{12} level <350 pg/mL. Prevalence of B_{12} deficiency was significantly lower for patients using a multivitamin (odds ratio, 0.31; 95% CI, 0.15–0.63).**

Conclusions: **Our results found a 22% prevalence of metabolically confirmed B_{12} deficiency in the primary care type 2 diabetic population. Although further research needs to be performed to determine the clinical implications of our findings, B_{12} deficiency should be considered in type 2 diabetic patients, especially those taking metformin. Furthermore, a daily multivitamin may protect against B_{12} deficiency. (J Am Board Fam Med 2009;22:528–534.)**

Type 2 diabetes is frequently treated by primary care physicians who must be able to manage both the disease and its multiple comorbidities. Vitamin B_{12} deficiency is a potential comorbidity that is often overlooked, despite the fact many diabetic patients are at risk for this specific disorder. For example, many diabetic patients are treated with metformin, a medication that lowers serum vitamin B_{12} levels[1–4] and is associated with vitamin B_{12} deficiency.[1,5–8] In addition, almost half of all diabetic patients are older than 60, an age group in which the prevalence of metabolically confirmed B_{12} deficiency ranges from 12% to 23%.[9–11] Because of these risk factors, defining the prevalence in the diabetic population may help determine whether primary care physicians should consider screening for B_{12} deficiency in diabetic patients. According to the World Health Organization's criteria for screening, a condition may be worthwhile to screen for if it is an important health problem and if there are tests available to detect the condition at an early, treatable stage.[12]

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One potential health problem from vitamin B_{12} deficiency is neuropathy, and almost 30% of diabetic patients older than 40 have impaired sensation in the feet.[13] Unfortunately, the symptoms of diabetic neuropathy overlap with the paresthesias, impaired vibration sense, and impaired proprioception associated with B_{12} deficiency.[14] As a result, B_{12} deficiency–induced nerve damage may be confused with or contribute to diabetic peripheral neuropathy.[15] Identifying the correct etiology of neuropathy is crucial because simple vitamin B_{12} replacement may reverse neurologic symptoms inappropriately attributed to hyperglycemia.

Vitamin B_{12} deficiency is traditionally diagnosed by laboratory findings of low serum vitamin B_{12} levels, typically in the setting of megaloblastic anemia. However, subclinical B_{12} deficiency often presents with normal serum B_{12} levels and hematologic parameters.[16] Elevated methylmalonic acid and homocysteine levels improve the diagnosis of tissue B_{12} deficiency[17–19] and may identify patients with deficiency at an early, reversible stage. Using these more specific diagnostic markers, we conducted a cross-sectional study to determine the extent of B_{12} deficiency in the diabetic population. Our primary aim was to define the prevalence of serologic B_{12} deficiency in the type 2 diabetic population. Our secondary aim was to assess for associated factors, such as age and metformin use, to help predict such deficiency.

Methods

Participants

This study used a descriptive, cross-sectional design to determine the prevalence of vitamin B_{12} deficiency among a sample of approximately 4000 type 2 diabetic patients cared for by the Madigan Army Medical Center (MAMC) Family Medicine Clinic (FMC). The MAMC Institutional Review Board approved the study.

Enrollment

Inclusion criteria were patients aged 45 years and older with the diagnosis of type 2 diabetes. The MAMC FMC appointment schedule was used daily to identify potential volunteers. Study personnel subsequently solicited diabetic patients presenting for a diabetic appointment for enrollment into the study. Diabetic patients that declined participation in the study were not tracked. Recruitment started in May 2005 and concluded in March 2006.

We obtained informed consent and asked those meeting the inclusion criteria to complete a questionnaire querying demographic information, medication and supplement use, and exclusion criteria. Patients were excluded if they had a history of pernicious anemia, chronic renal insufficiency defined by a creatinine >3.0, prior bariatric surgery, gastrectomy, B_{12} supplementation with B_{12} shots or an oral vitamin B_{12} dose of >500 mcg/day, prior ileum resection, or Crohn's disease. Medications and supplements queried were metformin, insulin (any form), other hypoglycemic medications, acid blockers (H_2 blockers and/or proton pump inhibitors), herbal supplements, multivitamins, and B-complex vitamins. Demographic information solicited were age, sex, and time of original diabetes diagnosis. We did not obtain dietary histories. The military Composite Health Care System database was cross-referenced to confirm metformin dosage and length of use.

Measurements

On enrollment, participants presented to the laboratory for phlebotomy. The individual blood samples were initially assayed within the MAMC laboratory for B_{12} and folate levels and then stored at −70°C. B_{12} levels <100 pg/mL were considered deficient whereas B_{12} levels >350 pg/mL were considered normal. Blood levels of B_{12} between 100 and 350 pg/mL were considered indeterminate[20–22] and the corresponding blood specimens were sent to Quest Diagnostics for determination of methylmalonic acid (reference range, 88–243 nmol/L) and homocysteine levels (reference range, 5.4–11.9 nmol/L). Specimens with methylmalonic acid and/or homocysteine levels above the upper limit of the laboratory reference range were considered elevated and indicative of B_{12} deficiency. Study staff notified and gave recommendations for treatment to all B_{12}-deficient patients and their primary care providers.

Statistical Analysis

Power analysis indicated that 187 to 232 patients were required to determine a 15% to 20% prevalence (with 95% CI of ±5%) of vitamin B_{12} deficiency in FMC type 2 diabetic patients.

All statistics were done with SPSS software version 12.0 (SPSS, Inc., Chicago, IL). Associations

Table 1. Characteristics of Outpatient Patients with Type 2 Diabetes

Characteristics (n = 195)	Value
Age (yrs) (mean [SD])	61.5 (9.6)
Serum B_{12} (pg/mL) (mean [SD])	470.0 (311.4)
Serum folate (ng/mL) (mean [SD])	17.7 (12.7)
Length of diabetes diagnosis (yrs) (mean [SD])	8.3 (7.2)
Body mass index (kg/m2) (mean [SD])	31.4 (6.6)
Hemoglobin A1c (mean [SD])	7.3 (1.2)
Male (n [%])	104 (53.3)
Current metformin use* (n [%])	133 (68.2)
Any history of metformin use (n [%])	157 (80.5)
Multivitamin use (n [%])	104 (53.3)
Acidblocker use (n [%])	70 (36.1)
Calcium use (n [%])	35 (17.9)
Race (n [%])	
White	110 (56.4)
Asian†	38 (19.4)
Black	24 (12.3)
Other‡	23 (11.8)
Metformin dose§ (mg) (n [%])	
1-1000	32 (24.1)
1001-1999	28 (21.0)
≥2000	73 (54.9)

*Current use defined as patients taking metformin upon enrollment in the study.
†Includes Pacific Islanders (n = 19).
‡Includes Hispanic (n = 4), Native American (n = 1), and unknown (n = 18).
§Numbers reflect patients currently taking metformin (n = 133).

between categorical values and B_{12} deficiency were done with χ^2 analysis. Associations between continuous variables and B_{12} deficiency were done with student's *t* test. Student's *t* test was also used to determine associations between metformin use and serum levels of B_{12}. A multivariate analysis using logistic regression was used to identify factors independently associated with B_{12} deficiency. Covariates chosen for the multivariate model were known or hypothesized biologic factors that would affect B_{12} deficiency.

Results

A total of 203 patients enrolled in the study. Eight patients were excluded: 2 had ileum resections, 2 were younger than 45 years of age, 2 were taking supplemental B_{12} >500 mcg/day, one had a gastrectomy, and one had Crohn's disease. The remaining 195 patients were included in the final analysis (Table 1).

Table 2. Bivariate Associations with B_{12} Deficiency

	B_{12} Deficiency		
Continuous Variables	Yes (n = 44)	No (n = 151)	*P*
Age, years (SD)	63.1 (9.4)	61.1 (9.6)	.22
Length of diabetes, years (SD)	10.3 (7.9)	7.8 (6.9)	.04
Categorical Variables (%)	Yes	No	
Gender			.62
Male	22.0	82.0	
Female	24.0	76.0	
Current metformin use*			.99
Yes	68.18	68.21	
No	31.82	31.79	
History of metformin use			.27
Yes	86.4	78.8	
No	13.6	21.2	
Acidblocker use			.45
Yes	40.9	34.7	
No	59.1	65.3	
Calcium use			.69
Yes	15.9	18.5	
No	84.1	81.5	
Multivitamin use			<.001
Yes	31.8	59.6	
No	68.2	40.4	

*Current metformin use as defined in Table 1.

Serum B_{12} levels ranged from 91 to 2818 pg/mL. Only one individual had a B_{12} level <100 pg/mL; 79 had intermediate levels between 100 and 350 pg/mL and required confirmatory testing to assess for deficiency. Forty-three of these patients (54%) with intermediate B_{12} levels had elevations of either methylmalonic acid or homocysteine, resulting in a total of 22% (n = 44) of patients diagnosed with metabolic B_{12} deficiency.

Table 1 shows the participants' demographic data. The majority of participants took metformin and approximately half were on a dose of ≥2000 mg daily at the time of enrollment. Approximately one-third of patients took either an H_2 blocker or a proton pump inhibitor, whereas just over half took a multivitamin daily (Table 2).

Table 2 shows key variable comparisons between patients with and without B_{12} deficiency. Metformin use, including both current use and history of metformin use, was not associated with B_{12} deficiency. On average, patients with a B_{12} deficiency suffered from diabetes for a significantly

longer time than patients without a B_{12} deficiency ($P = .04$). In addition, the percentage of patients identified as having a B_{12} deficiency was significantly lower among patients using a multivitamin compared with those patients who did not take a multivitamin supplement ($P < .001$). Only 4 patients reported taking B-complex vitamins, none of which had B_{12} deficiency.

When a second χ^2 was performed defining at risk for B_{12} deficiency as any serum B_{12} level <350 pg/mL, the results indicated that patients using metformin were statistically at higher risk for B_{12} deficiency ($P = .04$). A series of further analyses were subsequently performed to determine whether metformin dose was associated with B_{12} deficiency. In this study population, there was no statistically significant association between the average prescribed metformin dosage for patients with B_{12} deficiency (mean, 1776.32; SD, 578.06) and patients without B_{12} deficiency (mean, 1602.52; SD, 554.48), as shown by $t(155) = 1.67$ ($P = .09$).

A *t* test was conducted to determine whether any difference existed in the B_{12} levels of individuals currently using metformin versus those who were not. Patients taking metformin had statistically significant lower levels of B_{12} (425.99 pg/mL vs 527.49 pg/mL; $P = .01$).

Multivariate logistic regression analysis showed that in this study sample, use of a multivitamin significantly reduced odds of B_{12} deficiency (odds ratio, 0.31; 95% CI, 0.15–0.63). Use of H_2 blockers or proton pump inhibitors, age, and metformin use were not significantly associated with B_{12} deficiency.

Discussion

This is the first cross-sectional study we are aware of that was specifically designed to define the prevalence of B_{12} deficiency in patients with type 2 diabetes. In our cohort, we identified 22% of type 2 diabetic patients with B_{12} deficiency. Although it is possible to deduce the prevalence of B_{12} deficiency in diabetic patients from previous studies,[7] we feel our broad inclusion criteria provided an estimate that is more generalizable to the entire type 2 diabetic population. With a prevalence of 22%, physicians will need to consider the comorbid effects of B_{12} deficiency in a population already predisposed to neuropathic complications. More investigations are required to chart the full clinical impact of deficiency in these patients.

Despite that the clinical significance of a 22% prevalence of B_{12} deficiency in the diabetic population is unknown, the impact B_{12} deficiency may have as a cause of peripheral neuropathy in this population should be explored. Sixty percent to 70% of diabetic patients have mild to severe forms of nervous system damage,[13] the most common being peripheral neuropathy. The relatively high prevalence of B_{12} deficiency found in this study makes it likely that at least a portion of peripheral neuropathy cases in diabetic patients may be attributed to B_{12} deficiency. Previous studies have demonstrated that supplemental vitamin B_{12} improved somatic and autonomic symptoms of diabetic neuropathy.[23,24] Testing for, and treating, B_{12} deficiency in those patients with neuropathy may lead to improved clinical outcomes. Clinical trials are needed to further evaluate this link.

To improve the diagnosis of B_{12} deficiency, we measured methylmalonic acid and homocysteine blood levels. These markers of B_{12} deficiency have been shown to improve the sensitivity and specificity for detecting B_{12} deficiency. More importantly, these markers may discover tissue B_{12} deficiency in an early, asymptomatic, and reversible state. Other studies have used methylmalonic acid and homocysteine levels to define the prevalence of B_{12} deficiency in the elderly, but not in the diabetic population. These studies demonstrate a prevalence of B_{12} deficiency in the elderly that ranges from 12% to 23%.[9–11] Although the prevalence of B_{12} deficiency in our diabetic patients was in line with these results, it is important to note that the average age of our population was approximately 15 years younger than the average age of the elderly volunteers enrolled in these other studies. In addition, both bivariate and multivariate analyses demonstrated that age was not significantly associated with B_{12} deficiency. This suggests that type 2 diabetes, not age, may account for the 22% prevalence of B_{12} deficiency. Unfortunately, the ability to compare our prevalence results to the general population is limited because there are no published studies that examine the prevalence of B_{12} deficiency in the general population using methylmalonic acid and homocysteine levels for diagnosis.

Patients on chronic metformin therapy seem to be at increased risk for B_{12} deficiency. Its use is associated with lower serum vitamin B_{12} levels[1–4,6] and megaloblastic anemia.[1] Several studies associate metformin use with established clinical B_{12} de-

ficiency.[1,5,7] In fact, higher doses and longer treatment with metformin seem to be risk factors for such deficiency.[25] Although we found that patients using metformin had lower B_{12} levels, we did not find metformin use to be associated with overt B_{12} deficiency. In addition, there were no statistically significant metformin dose-dependent relationships, despite a trend between higher dosages of metformin use and B_{12} deficiency. Our study was not designed nor powered to find these secondary associations. As part of a post hoc analysis we did look at diabetic patients at risk for B_{12} deficiency to determine whether there was a significant association with metformin use. Current metformin use was associated with a significantly higher risk for B_{12} deficiency when defined as a serum B_{12} level <350 pg/mL. Patients with B_{12} levels <350 pg/mL may be at risk for B_{12} deficiency becuase tissue deficiency may occur despite normal serum B_{12} levels.[10,20–22] Identification of patients "at risk" for B_{12} deficiency as those with serum B_{12} <350 pg/mL may help the clinician define a level to test for B_{12} deficiency using specific tissue markers, especially among diabetics who are using metformin.

Our multivariate analysis looked for specific associations for B_{12} deficiency. We entered into our model known and potential risk or protective factors for B_{12} deficiency. Multivitamin use seemed to protect diabetic patients from B_{12} deficiency. The effect of multivitamins raising serum B_{12} levels is documented in the literature. Randomized trials in adults taking 6 to 9 mcg of B_{12} daily show effects of higher serum B_{12} levels compared with placebo.[26,27] There are no known studies specifically evaluating whether a daily multivitamin prevents B_{12} deficiency. Our findings may be noteworthy because conventional treatment of B_{12} deficiency is with high dose oral supplementation or B_{12} injections.[28] Yet, most multivitamin formulations typically contain 6 to 25 mcg of supplemental B_{12},[29,30] which seemed to be enough to protect against B_{12} deficiency in type 2 diabetic patients. Further research needs to be conducted before the validity of multivitamin use to prevent against B_{12} deficiency is confirmed. Other factors known to increase risk for B_{12} deficiency, such as advanced age and acid blocker use, were not significantly associated with B_{12} deficiency.

There were several limitations to our study. Our cohort was a sample population from a military health care system. MAMC has been recognized as an innovator in the chronic care model of diabetes. Subsequently, most patients had well-controlled diabetes. In populations with poorer glycemic control, our study may not be generalizable because the rates of metformin use may not be similar. However, a population with poorer glycemic control is at higher risk for neuropathy and may benefit even more from early identification of concomitant B_{12} deficiency. In addition, we only included diabetics older than 45, consistent with the age group of the majority of the type 2 diabetic population in the United States.[13] Overall, we feel our participants represent typical primary care diabetic patients who present to an outpatient clinic for ongoing care.

The cross-sectional nature of our study limits us to describing a population. Therefore, the findings relevant to our secondary aims were limited to associations. The primary objective, however, was to define the prevalence of B_{12} deficiency in the diabetic population, for which a cross-sectional study is appropriate. Additional studies will be needed to prove causation. In addition, defining B_{12} deficiency based solely on biochemical markers remains controversial.[31–33] Although elevations in methylmalonic acid have been correlated with clinical manifestations of B_{12} deficiency,[17,18] we did not evaluate for evidence of megaloblastic anemia or neuropathic disease. Therefore, the clinical significance of metabolically confirmed B_{12} deficiency in our patient group is unknown. Finally, the B_{12}-deficient patients identified in our study were not followed for treatment effect with supplemental vitamin B_{12} or to evaluate for normalization of B_{12} and methylmalonic acid levels. Such follow-up would have helped confirm the diagnosis of vitamin B_{12} deficiency.

Conclusion

Finding a prevalence of 22% raises the question of whether to screen for B_{12} deficiency in all patients. No studies that we are aware of have looked at outcomes for treating asymptomatic B_{12} deficiency, so the benefit of such treatment is unknown. Primary care physicians should recognize that up to one fifth of their diabetic patients may have B_{12} deficiency; they should consider B_{12} deficiency in the differential diagnosis when managing comorbidities of diabetes, especially neuropathy. Further studies need to be undertaken to determine whether screening and subsequent treatment can

prevent peripheral neuropathy from developing in patients with type 2 diabetes before advocating for universal screening. Finally, empiric treatment with a multivitamin should also be explored as a method for reducing the incidence of B_{12} deficiency in the type 2 diabetic population.

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