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ARTICLE

Vitamin B12 deficiency in patients with diabetes at a specialised diabetes clinic, Botswana

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Objective: To estimate the prevalence of Vitamin B12 deficiency among patients with diabetes.
Methodology: This cross-sectional study was undertaken on 351 patients with diabetes at a specialised public diabetes clinic in Gaborone between July 2017 and October 2017. Clinical, anthropometry and laboratory data were collected. Vitamin B12 deficiency was defined by levels < 150 pmol/l.
Results: The mean (SD) age of the participants was 57 (15) years, two-thirds (67.2%) were females, and the majority (92.9%) had Type 2 diabetes. Most (89.5%) participants were on metformin. The prevalence of vitamin B12 deficiency was 6.6%. Compared with participants with normal Vitamin B12 levels, deficient participants were significantly older (64 vs. 56 years, $p = 0.014$) and had a longer duration of metformin use (7 vs. 4 years, $p = 0.024$). The use of acid blockers was also associated with vitamin B12 deficiency ($p = 0.012$). There was no difference in the prevalence of peripheral neuropathy between those with normal and deficient vitamin B12 levels.
Conclusion: Vitamin B12 deficiency exists among patients with diabetes in the setting discussed. Regular vitamin B12 assessment may be beneficial, especially among diabetes patients who are old, those taking metformin over a long duration and patients on acid blockers.

Keywords: Botswana, diabetes mellitus, metformin, vitamin B12 deficiency, prevalence

Introduction

Vitamin B12 is a water-soluble vitamin involved in DNA synthesis, haematopoiesis and neurological function.[1] The prevalence of vitamin B12 deficiency in diabetes mellitus (DM) ranges between 5.8% and 52%.[2–8] The association of vitamin B12 deficiency and Type 2 diabetes (T2DM) is mainly due to metformin's long-term use, as demonstrated by evidence from both observational and interventional studies.[1,8] This association's exact mechanism is unknown but has been ascribed to intestinal malabsorption of vitamin B12 due to metformin.[9,10] Pernicious anaemia, which is characterised by an autoimmune-mediated chronic atrophic gastritis, is a classically described cause of vitamin B12 deficiency among type 1 diabetes patients.[11] As vitamin B12-related hematologic and neuropsychiatric disorders are reversible by early diagnosis, regular assessment of vitamin B12 levels is essential.[2] Prompt treatment may be important in patients with diabetes.[12] As the prevalence of vitamin B12 deficiency in patients with diabetes in Botswana is unknown, this study was undertaken to estimate the prevalence of Vitamin B12 deficiency among patients with diabetes.

Methods

Study design and participants

We conducted a cross-sectional study among patients attending Princess Marina diabetes clinic in Gaborone between July 2017 and October 2017. The clinic has been operational since 2011 as a referral centre for health facilities in Gaborone and nearby towns. Eligible patients were males and females, aged ≥ 18 years, diagnosed with type 1 and type 2 diabetes mellitus. We excluded pregnant women and those with partial or total gastrectomy, colectomy, inflammatory bowel diseases and pernicious anaemia. An estimated sample size of 354 patients was calculated. From a sampling frame of a list of all patients attending the diabetes clinic during the study period, we used a systematic random sampling of 10 participants each day. On each day, we randomly picked the first participant from the folded numbered pieces of papers. Subsequently, every fifth patient in the clinic attendees was selected until the day's desired sample size was reached.

Data collection and procedures

We used a researcher-administered questionnaire to obtain information on patients' age, sex, HIV status, diabetes type and diabetes duration. We also inquired about the use of metformin, antacids, oral contraceptives and calcium supplements. For metformin, the daily dose of metformin and duration of metformin use was recorded We calculated the body mass index (BMI) as weight in kilograms (kg) divided by height in metres squared, and categorised participants with a BMI ≥ 25 kg/m^2 and ≥ 30 kg/m^2 as overweight and obese, respectively.[13] Peripheral neuropathy was defined by a score > 6 in the Neuropathy Total Symptom Score-6 (NTSS-6) questionnaire.[14] We assessed serum B12 levels, haemoglobin (Hb), mean corpuscular volume (MCV) and glycated haemoglobin (HbA1c) in all participants. We defined vitamin B12 deficiency as serum B12 levels < 150 pmol/l.[15,16] Anaemia was defined as Hb < 13 g/dl for males and < 12 g/dl for females.[17] Macrocytosis was characterised as mean corpuscular volume (MCV) > 100 fl.19.[18]

Statistical analysis

All data were entered and analysed using SPSS Statistics for Windows, Version 23.0 (IBM SPSS Statistics for Windows, Version 23.0 [IBM Corp, Armonk, NY, USA]). We presented

continuous variables as mean (standard deviation [SD]) or median (interquartile range [IQR]) as appropriate. Categorical variables appear as counts and percentages. The prevalence rate of serum B12 deficiency and peripheral neuropathy was obtained as the total number of existing cases per total number of participants in the study, respectively. Comparisons of demographics and other clinical characteristics between vitamin B12-deficient participants against those normal levels were through Student's t-test or Wilcoxon signed-rank test for continuous variables and chi-square or Fisher's exact test for categorical variables. A *p*-value of less than 0.05 was considered significant.

Results

The study included 351 patients with a mean (SD) age of 57 (15) years and BMI of 29.49 (5.9) kg/m^2 (Table 1). The majority were females (67.2%) and those with type 2 diabetes (92.9%). The median (IQR) duration since the diagnosis of diabetes was 6 (2–12) years, and 89.5% of participants were on metformin. The overall median (IQR) HbA1c and haemoglobin were 7.4 (6.4–9.2) % and 3 g/dl.

Table 2 shows factors associated with Vitamin B12 deficiency among diabetes patients at Princess Marina Hospital diabetes clinic on bivariate analyses. Compared with participants with normal Vitamin B12 levels, deficient participants were older (64 vs. 56 years, $p = 0.014$), had longer metformin use duration (7 vs. 4 years, $p = 0.024$), had low haemoglobin (13.4 vs. 12.8; $p = 0.038$) and used acid blockers ($p = 0.012$).

Table 1: Baseline characteristics of patients with diabetes at Princess Marina hospital diabetes clinic, between July 2017 and October 2017 ($n = 351$)

Characteristic	Mean (SD)
Gender, *n* (%):	
Male	115 (32.8)
Female	236 (67.2)
Age (years), mean, SD:	57 (15)
BMI (kg/m^2), mean, SD:	29.49 (5.9)
Diabetes type, *n* (%):	
type 1	25 (7.1)
type 2	326 (92.9)
Diabetes duration (years), median (IQR)	6 (2–12)
Medications, *n* (%):	
Insulin	121 (34.5)
Acid blocker	90 (25.6)
Calcium supplements	26 (7.4)
Oral contraceptive (Yes)	4 (1.1)
ARV treatment (Yes)	40 (11.1)
Metformin	314 (89.5)
Type 1 diabetes	0
Type 2 diabetes	314 (89.5)
Metformin dose, *n* (%):	
1–1 000 mg	74 (21.1)
1 001–2 000 mg	149 (42.5)
> 2 000 mg	91 (25.9)
Laboratory:	
MCV(FL), mean (SD)	84.4 (7.4)
Haemoglobin(g/dl), median (IQR)	13.3 (12.4–14.3)
HbA1c (%), median (IQR)	7.4 (6.4–9.2)
Vitamin B12 levels (pmol/l), median (IQR)	322 (228–426)

Discussion

This outpatient cross-sectional study found 6.6% of patients with diabetes at Princess Marina Hospital diabetes clinic to be vitamin B12 deficient. The deficiency was significantly common with increased age, long duration of metformin use, and acid blockers. There was no difference in the prevalence of peripheral neuropathy in those with normal vitamin B12 levels and those with vitamin B12 deficiency.

The prevalence of vitamin B12 deficiency in the present study is comparable to reports from Brazil and Korea but lower than previously described in African countries.[8,19–21] For instance, the prevalence of vitamin B12 deficiency in South Africa and Uganda was (28%) and (10.7%), respectively.[20,21] The variations in diets across communities may partly explain the differences in the prevalence of vitamin B12 deficiency across studies. The high consumption of meat among the Botswana population may explain the lower prevalence of vitamin B12 in our population than in other African settings. Meat consumption in Botswana increased by 0.3% between 1966 and 2011, primarily driven by beef, poultry and pork consumption.[22] The variation in the cut-off values for vitamin B12 deficiency across studies may also explain the wide variations in B12 deficiency from 5.8% to 52% in studies worldwide.[5,6,8,10,19,20,23–25] In some studies, individuals with borderline vitamin B12 levels were further screened for evidence of elevated serum methylmalonic acid or homocysteine concentrations.[2] In this way, patients with borderline vitamin B12 levels were further categorised into those with or without vitamin B12 deficiency. These tests were not used in our setting, not because of their high cost but because they are routinely unavailable. Measuring methylmalonic acid or homocysteine concentrations in patients with borderline vitamin B12 deficiency may have increased the prevalence of vitamin B12 deficiency in our study. Lastly, genetic and environmental differences could be the other factors that explain the variation in the prevalence of vitamin B12 deficiency in different settings.[26] Several single-nucleotide polymorphisms (SNPs) in multiple genes interact with environmental factors to cause vitamin B12 deficiency.[27]

Consistent with previous studies, metformin use was associated with vitamin B12 deficiency in our patients.[19] In the present study, vitamin B12 deficient patients had a longer duration of metformin use, as reported in other studies.[19,28] Levels of serum B12 are inversely associated with the dose and duration of metformin use.[5,10,28–31] Reports have shown a decrease in vitamin B12 levels as early as four months after initiation of metformin.[10,31] The exact mechanism of this association is unknown but is ascribed to the binding of the hydrophobic tail of biguanide to the hydrocarbon core of membranes. The biguanide group positively charges the membrane and can displace divalent cations such as calcium.[9] As a result, metformin impairs the calcium-dependent uptake of vitamin B12 into the ileal cells.[10]

The use of acid blockers was significantly associated with vitamin B12 deficiency, consistent with previous studies. In a case-control study among Medicaid patients in the USA, vitamin B12 deficiency was found in 18% of patients using acid blockers compared with 11% in the control group.[32] Other previous studies have, however, found no significant association between long-term proton pump inhibitor use and vitamin B12 status.[8,33] The connection between vitamin B12 deficiency and proton pump inhibitors or H2 blockers

Table 2: Factors associated with Vitamin B12 deficiency among patients with diabetes at Princess Marina Hospital diabetes clinic, between July 2017 and October 2017

	B12 deficiency		
Characteristic	**Yes (*n* = 23)**	**No (*n* = 328)**	***p*-value**
Female, *n* (%)	17 (73.9)	219 (66.8)	0.498
Diabetes type 2, *n* (%)	21 (91.3)	305 (93.0)	0.762
Diabetes type 1, *n* (%)	0	25 (7.6)	
Metformin use, *n* (%)	21 (91.3)	293 (89.3)	0.766
Metformin dose category, *n* (%):			
1–1 000 mg	2 (9.5)	72 (24.6)	0.455
1 001–2 000 mg	12 (57.1)	137 (46.8)	
> 2 000 mg	7 (33.3)	84 (28.7)	
Insulin use, *n* (%)	8 (6.6)	113 (93.4)	0.974
Acid blocker use, *n* (%)	11 (47.8)	79 (24.1)	**0.012**
Calcium supplement, *n* (%)	4 (17.4)	22 (6.7)	0.059
Oral contraceptives use, *n* (%)	0	4 (1.2)	0.594
HIV status, *n* (%):			
Positive	1 (4.3)	44 (13.4)	< **0.001**
Negative	14 (60.9)	258 (78.7)	
Unknown	8 (34.8)	26 (7.9)	
ARV treatment, *n* (%)	0	40 (12)	0.075
Macrocytosis (present), *n* (%)	0	5 (1.5)	0.548
Anemia, *n* (%)	8 (38.8)	59 (18.3)	0.53
Peripheral neuropathy (present), *n* (%)	9 (39.1)	82 (25)	0.135
Age (years), mean (SD)	64 ± 13.2	56 ± 14.5	**0.014**
BMI (kg/m^2), mean (SD)	29.94 ± 6.5	29.46 ± 5.8	0.708
MCV(FL), mean (SD)	85.9 ± 8.9	84.29 ± 7.2	0.387
Diabetes duration (years), median (IQR)	7 (5–7)	6 (2–12)	0.269
Duration of metformin use (years), median (IQR)	7 (4–7)	4 (1–10)	**0.024**
Haemoglobin (g/dl), median (IQR)	12.8 (11.4–13.5)	13.4 (12.5–14.4)	**0.038**
HbA1c (%), median (IQR)	7.2 (6.5–8.8)	7.4 (6.4–9.2)	0.829
Vitamin B12 levels (pmol/l), median (IQR)	126 (117–138)	333 (246–443)	< **0.001**

(acid blockers) is primarily due to decreased gastric acidity, contributing to vitamin B12 malabsorption.[34] Both the acid blockers and proton pump inhibitors cause a reduction in secretion and discharge of acid discharges by the parietal cells, which are essential for the breakdown of vitamin B12 from nutritional sources.[10,34,35] In the absence of gastric acid, vitamin B12 would not be cleaved from dietary protein and would not bind to R-proteins. The latter protects vitamin B12 from pancreatic digestion.[36]

In this study, participants with vitamin B12 deficiency were significantly older than those with normal vitamin B12 levels. The prevalence of vitamin B12 deficiency increases with age due to either inadequate dietary intake or impaired vitamin B12 absorption.[37] Ageing is associated with decreases in taste acuity and smell, deteriorating dental health and a reduction of physical activity, which may all affect nutrient intake.[38] Insufficient nutritional intake of vitamin B12 accounts for about 2% of the vitamin B12 deficiency in the elderly, especially those living in institutions.[39] Vitamin B12 malabsorption is the most frequent cause of vitamin B12 deficiency in the elderly, accounting for about 40% to 70% of cases.[39,40] It is caused by atrophic gastritis that occurs in 20% to 50% of the elderly population. Gastritis is due to either *Helicobacter pylori* (*H. pylori*) infection or autoimmune mechanisms.[41,42] *H. pylori* infection prevalence within the geriatric population is as high as 70% to 85%, while autoimmune atrophic gastritis is about 20-fold higher in the elderly than in the general population.[43] Another reason for the high prevalence of vitamin B12 deficiency in the elderly is the high rate of acid blocker prescriptions.[39,40]

In the current study, increased but low haemoglobin levels were significantly associated with vitamin B12 deficiency. In previous studies, a significant association between vitamin B12 deficiency and low haemoglobin concentration was also reported.[44,45] Inadequate vitamin B12 results in ineffective DNA synthesis and impaired erythropoiesis, which may account for significantly low haemoglobin among vitamin B12 deficient patients.[46]

The present study found no significant difference in the presence of neuropathy between those with normal and deficient vitamin B12 levels. Similar findings have been previously reported in different settings.[20,47–49] In the current study, NTSS-6 was used to assess neuropathy. The tool is prone to subjectivity as it is symptom dependent, and there is a possibility of misclassification of participants. Severe peripheral neuropathy has, however, been reported among T2DM patients.[50–52] This is common among patients on metformin due to drug-induced vitamin B12 deficiency.[50,53] Vitamin B12 deficiency causes elevated homocysteine and methylmalonic acid, which has been shown to have potentially toxic effects on neurons.

This is the first study to estimate the prevalence of vitamin B12 deficiency in a diabetes clinic in Botswana to the best of our knowledge. However, our findings should be interpreted while considering several limitations. We assessed vitamin B12 status using serum vitamin B12 levels. Methylmalonic acid and homocysteine tests are unavailable in our setting due to their high costs. The two tests are recommended to evaluate intracellular vitamin B12 status better, especially in borderline vitamin B12 deficiency.[54] Parietal cell and intrinsic factor antibodies status were not correlated with the confirmed vitamin B12-deficient patients to exclude them from this study due to the unavailability of antibody tests in our local setting. The study does not explore the relationship between various factors and vitamin B12 deficiency, including dietary details, a vegan diet, alcohol history, use of multivitamins etc. The duration of metformin use is dependent on the patient's history and thus subject to recall bias. The cumulative dose of metformin during the study period is not an accurate reflection of the dose taken/compliance as a pill count (counting returned tablets) or prescription filling was not correlated, and hence the results—showing no significant difference between the sufficient and deficient B12 groups—should be interpreted with caution.

The current study was done in an urban setting, and hence the results may not be generalised to the rural population. Also, a single-centre nature of the study limits the generalisation of the study findings. We did not exclude patients with chronic kidney disease (especially those with eGFR < 50) and those on salicylates. Both are confounders for vitamin B12 deficiency as they are known to decrease the excretion rate of cyanocobalamin which could result in a higher serum level. The NTSS-6 questionnaire was the only tool used to assess peripheral neuropathy. Although it is validated for evaluating peripheral neuropathy, it is prone to subjectivity as it is symptom dependent.[14] Modified Toronto Clinical Neuropathy Score (m-TCNS) with better reliability is an alternative in subsequent studies.[55]

In conclusion, the study demonstrated that vitamin B12 deficiency exists among diabetes patients attending Princess Marian Hospital diabetes clinic. Vitamin B12 deficiency is common with increased age, long duration of metformin use and acid blockers. We recommend screening and treating vitamin B12 deficiency in patients with diabetes who are on long-term metformin and acid blockers.

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