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.9 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/m² and ≥ 30 kg/m² as overweight and obese, respectivel y.11 Peripheral neuropathy was defined by a score > 6 in the Neuropathy Total Symptom Score-6 (NTSS-6) questionnaire.11 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/m² (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).

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. 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. 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. 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. In some studies, individuals with borderline vitamin B12 levels were further screened for evidence of elevated serum methylmalonic acid or homocysteine concentrations. 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. Several single-nucleotide polymorphisms (SNPs) in multiple genes interact with environmental factors to cause vitamin B12 deficiency.

Consistent with previous studies, metformin use was associated with vitamin B12 deficiency in our patients. In the present study, vitamin B12 deficient patients had a longer duration of metformin use, as reported in other studies. Levels of serum B12 are inversely associated with the dose and duration of metformin use. Reports have shown a decrease in vitamin B12 levels as early as four months after initiation of metformin. 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. As a result, metformin impairs the calcium-dependent uptake of vitamin B12 into the ileal cells.

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. Other previous studies have, however, found no significant association between long-term proton pump inhibitor use and vitamin B12 status. The connection between vitamin B12 deficiency and proton pump inhibitors or H2 blockers

| Characteristic | Mean (SD) |
|---------------|-----------|
| Gender, n (%): |           |
| Male          | 115 (32.8) |
| Female        | 236 (67.2) |
| Age (years), mean, SD: | 57 (15) |
| BMI (kg/m²), 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 | 314 (89.5) |
| Type 2 diabetes | 0          |
| 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) |
Vitamin B12 malabsorption is the most frequent cause of vitamin B12 deficiency in the elderly, especially those with low haemoglobin levels. Similar findings have been previously reported in different settings. 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. This is common among patients on metformin due to drug-induced vitamin B12 deficiency. Vitamin B12 deficiency causes elevated homocysteine and methylmalonic acid, which has been shown to have potentially toxic effects on neurons.

In this study, participants with vitamin B12 deficiency were significantly older than those with normal vitamin B12 levels. The present study found no significant difference in the prevalence of neuropathy between those with normal and deficient vitamin B12 levels. Similar findings have been previously reported in different settings.

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

| 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 (24.8)   | 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 (34.3)  | 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)    |         |
| 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/m²), mean (SD) | 29.18 ± 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   |
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. Methylnalonic 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.\textsuperscript{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.\textsuperscript{18} Modified Toronto Clinical Neuropathy Score (m-TCNS) with better reliability is an alternative in subsequent studies.\textsuperscript{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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**References**

1. Kibirige D, Mweebaze R. Vitamin B12 deficiency among patients with diabetes mellitus: is routine screening and supplementation justified? J Diab Metab Dis. 2013;12(1):17.
2. Pflipsen MC, Oh RC, Sagull A, et al. The prevalence of vitamin B12 deficiency in patients with type 2 diabetes: a cross-sectional study. J Am Board Family Med. 2009;22(5):528–34.
3. Reinstatler L, Qi YP, Williamson RS, et al. Association of biochemical B12 deficiency with metformin therapy and vitamin B12 supplementation: the national health and nutrition examination survey. 1999–2006. Diab Care. 2012;35(2):327–33.
4. Qureshi SA, Ainsworth A, Winocour PH. Metformin therapy and assessment for vitamin B12 deficiency: is it necessary? Pract Diab. 2011;28(7):302–4.
5. De Jager J, Kooij A, Lehert P, et al. Long term treatment with metformin in patients with type 2 diabetes and risk of vitamin B12 deficiency: randomised placebo controlled trial. Br Med J. 2010;340:c2181.
6. Sparre Hermann L, Nilsson B, Wette S. Vitamin B12 status of patients treated with metformin: a cross-sectional cohort study. Br J Diab Vascular Dis. 2004;4(6):401–6.
7. Liu K, Dai D, Ho W, et al. Metformin-associated vitamin B12 deficiency in the elderly. Asian J Gerontol Geriatr. 2011;6(2):82–87.
8. Nervo M, Lubini A, Raimundo FV, et al. Vitamin B12 in metformin-treated diabetic patients: a cross-sectional study in Brazil. Rev Assoc Med Bras. 2011;57(1):46–9.
9. Liu K, Dai D, Ho W, et al. Metformin-associated vitamin B12 deficiency in the elderly. Asian J Gerontol Geriatr. 2011;6:82–7.
10. Bauman WA, Shaw S, Jayatilake E, et al. Increased intake of calcium reverses vitamin B12 malabsorption induced by metformin. Diabetes Care. 2000;23(9):1227–31.
11. De Block CE, Devlieghere I, Van Gaal LF. Autoimmune gastritis in type 1 diabetes: a clinically oriented review. J Clin Endocrinol Metab. 2008;93(2):363–71.
12. Langan RC, Zawistowski KJ. Update on vitamin B12 deficiency. Am Fam Physician. 2011;83(12):384–389.
13. Romero-Corral A, Somers VK, Sierra-Johnson J, et al. Accuracy of body mass index in diagnosing obesity in the adult general population. Int J Obes. 2008;32(6):959–66.
14. Bastry EJ, Price KL, Bril V, et al. Development and validity testing of the neuropathy total symptom score-6: questionnaire for the study of sensory symptoms of diabetic peripheral neuropathy. Clin Ther. 2005;27(8):1278–94.
15. Chanarin I, Metz J. Diagnosis of cobalamin deficiency: the old and the new. Br J Haematol. 1997;97(4):695–700.
16. de Benoist B. Conclusions of a WHO technical consultation on folate and vitamin B12 deficiencies. Food Nutr Bull. 2008;29(2 Suppl):S238–44.
17. Beutler E, Waalen J. The definition of anaemia: what is the lower limit of normal of the blood hemoglobin concentration? Blood. 2006;107(5):1747–50.
18. Cell MRB, Cell MRB, Cell NRB. Vitamin B12 Deficiency. 1999.
19. Ko S-H, Ko S-H, Ahn Y-B, et al. Association of vitamin B12 deficiency and metformin use in patients with type 2 diabetes. J Korean Med Sci. 2014;29(7):965–72.
20. Ahmed MA. Metformin and vitamin B12 deficiency: where do we stand? J Pharm Pharm Sci. 2016;19(3):382–98.
21. Akabaw GP, Kibirige D, Mugenyi L, et al. Vitamin B12 deficiency among adult diabetic patients in Uganda: relation to glycaemic control and haemoglobin concentration. J Diab Metab Dis. 2015;15(1):26.
22. Setchugato N. Food consumption changes in Botswana since 1966. Pula Botswana J Afr Stud. 2017;31(2):87–94.
23. Liu KW, Dai LK, Jean W. Metformin-related vitamin B12 deficiency. Age Ageing. 2006;35(2):200–1.
24. Beulens JW, Hart HE, Kuila R, et al. Influence of duration and dose of metformin on cobalamin deficiency in type 2 diabetes patients using metformin. Acta Diabetol. 2015;52(1):47–53.
25. Nasser F, Islam T. Association of vitamin B12 and metformin in type II diabetes patients in Bahrain. J Diabetes Metab. 2018;9(6):1–6.
26. Surendran S, Adaikakoteswari A, Saravanan P, et al. An update on vitamin B12-related gene polymorphisms and B12 status. Genes Nutr. 2018;13(1):2.
27. Haggarty P. B-vitamins, genotype and disease causality: symposium on genetic polymorphisms and disease ‘risk’. Proc Nutr Soc. 2007;66(4):339–47.
28. Akinlade K, Agbебaku S, Rahamon S, et al. Vitamin B 12 levels in patients with type 2 diabetes mellitus on metformin. Ann Ib Postgrad Med. 2015;13(2):79–83.
29. Tomkin G, Hadden D, Weaver J, et al. Vitamin-B12 status of patients on long-term metformin therapy. Br Med J. 1971;2(5763):685–8.
30. Ting RZ-W, Szeto CC, Chan MH-M, et al. Risk factors of vitamin B12 deficiency in patients receiving metformin. Acta Diabetol. 2015;52(1):47–53.
31. Nasser F, Islam T. Association of vitamin B12 and metformin in type II diabetes patients in Bahrain. J Diabetes Metab. 2018;9(6):1–6.
32. Akinlade K, Agbебaku S, Rahamon S, et al. Vitamin B12 levels in patients with type 2 diabetes mellitus on metformin. Ann Ib Postgrad Med. 2015;13(2):79–83.
33. Den Elzen W, Groeneveld Y, De Ruijter W, et al. Long-term use of proton pump inhibitors and vitamin B12 status in elderly individuals. Aliment Pharmacol Ther. 2008;27(6):491–7.
34. Valuck RJ, Ruscin JM. A case-control study on adverse effects: H2 blocker or proton pump inhibitor use and risk of vitamin B12 deficiency in older adults. J Clin Epidemiol. 2004;57(4):422–8.
35. Ruscin JM, Lee Page R, Valuck RJ. Vitamin B12 deficiency associated with histamine2-receptor antagonists and a proton-pump inhibitor. Ann Pharmacother. 2002;36(5):812–6.
36. Festen H. Intrinsic factor secretion and cobalamin absorption: physiology and pathophysiology in the gastrointestinal tract. Scand J Gastroenterol. 1991;26(sup188):1–7.
37. Wong C. Vitamin B12 deficiency in the elderly: is it worth screening. Hong Kong Med J. 2015;21(2):155–64.
38. Gariballa S, Sinclair A. Nutrition, ageing and ill health. Br J Nutr. 1998;80(1):7–23.
39. Andrès E, Loukili NH, Noel E, Kaltenbach G, Abdelgheni MB, Perrin AE, et al. Vitamin B12 (cobalamin) deficiency in elderly patients. Can Med Assoc J. 2004;171(3):251–9.
40. Carmel R. Cobalamin (vitamin B12). Modern Nutr Health Dis. 2006;10:482–97.
41. Malfertheiner P, Sipponen P, Naumann M, et al. Helicobacter pylori eradication has the potential to prevent gastric cancer: a state-of-the-art critique. Am J Gastroenterol. 2005;100(9):2100.
42. Benberin V, Bektayeva R, Karabayeva R, et al. Prevalence of H. pylori infection and atrophic gastritis among symptomatic and dyspeptic adults in Kazakhstan. A hospital-based screening study using a panel of serum biomarkers. Anticancer Res. 2013;33(10):4595–602.
43. Regev A, Fraser GM, Braun M, et al. Seroprevalence of Helicobacter pylori and length of stay in a nursing home. Helicobacter. 1999;4(2):89–93.
44. Kwok T, Cheng G, Woo J, et al. Independent effect of vitamin B12 deficiency on hematological status in older Chinese vegetarian women. Am J Hematol. 2002;70(3):186–90.
45. Soofi S, Khan GN, Sadiq K, et al. Prevalence and possible factors associated with anaemia, and vitamin B12 and folate deficiencies in women of reproductive age in Pakistan: analysis of national-level secondary survey data. BMJ Open. 2017;7(12):e018007.
46. Thompson WG, Babitz L, Cassino C, et al. Evaluation of current criteria used to measure vitamin B12 levels. Am J Med. 1987;82(2):291–4.
47. Biemans E, Hart HE, Rutten GE, et al. Cobalamin status and its relation with depression, cognition and neuropathy in patients with type 2 diabetes mellitus using metformin. Acta Diabetol. 2015;52(2):383–93.
48. Raizada N, Jyotsna VP, Sreenivas V, et al. Serum vitamin B12 levels in type 2 diabetes patients on metformin compared to those never on metformin: A cross-sectional study. Indian J Endocrinol Metab. 2017;21(3):424.
49. Ott S, Oznas O. Investigation of the vitamin B12 deficiency with peripheral neuropathy in patients with type 2 diabetes mellitus treated using metformin. Northern Clinics of Istanbul. 2017;4(3):233.
50. Wile DJ, Toth C. Association of metformin, elevated homocysteine, and methylmalonic acid levels and clinically worsened diabetic peripheral neuropathy. Diabetes Care. 2010;33(1):156–61.
51. Niafar M, Hai F, Porhomayon J, et al. The role of metformin on vitamin B12 deficiency: a meta-analysis review. Intern Emerg Med. 2015;10(1):93–102.
52. Groenier K, Houweling S, Bilo H, et al. Vitamin B12 deficiency and the lack of its consequences in type 2 diabetes patients using metformin. Neth J Med. 2013;71(7):386–90.
53. Healton EB, Savage DG, Brust J, et al. Neurologic aspects of cobalamin deficiency. Medicine (Baltimore). 1991;70(4):229–45.
54. Yetley EA, Pfeiffer CM, Phinney KW, et al. Biomarkers of vitamin B12 status in NHANES: a roundtable summary. Am J Clin Nutr. 2011;94(1):3135–21S.
55. Bril V, Tomioka S, Buchanan R, et al. Reliability and validity of the modified Toronto clinical neuropathy score in diabetic sensorimotor polyneuropathy. Diabetic Med. 2009;26(3):240–6.

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