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Vitamin B_{12} deficiency in type 2 diabetic patients on metformin: a cross-sectional study from South-Western part of Ghana

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ABSTRACT

Introduction: Metformin is the most widely administered anti-diabetic medication among type 2 diabetes mellitus (T2DM) patients. However, metformin induces vitamin B12 malabsorption which may increase the risk of vitamin B12 deficiency among T2DM patients. We determined the prevalence of vitamin B12 deficiency and related risk factors among Ghanaian T2DM patients on metformin therapy.

Methods: This cross-sectional study recruited 196 T2DM patients attending the outpatient diabetic clinic at the Effia Nkwanta Regional Hospital, Ghana. Fasting venous blood was collected for biochemical analysis. Vitamin B12 deficiency was defined as serum B12 <100 pg/ml and methylmalonic acid (MMA) ≥ 0.4µmol/L.

Results: The prevalence of vitamin B12 deficiency based on serum vitamin B12, MMA, and the combination of both methods were 32.1%, 14.8%, and 14.3%, respectively. Longer duration of metformin use [5-9 years; aOR= 2.83, 95% CI (1.03-7.81), p=0.045 and ≥10 years; aOR= 4.17, 95% CI (1.41-12.33), p=0.010], higher daily dose of metformin [1000-2000 mg/day; aOR= 1.34, 95% CI (0.25-2.74), p=0.038 and >2000 mg/day; aOR= 1.13, 95% CI (0.39-2.97), p=0.047], and very high body fat [aOR= 2.98, 95% CI (1.47-6.05), p=0.020] were significantly associated with increased odds of vitamin B12 deficiency. For daily dose of metformin, a cutoff value of 1500 mg/day presented with a sensitivity, specificity, and AUC of 71.4%, 40.1%, and 0.54 (95% CI, 0.53-0.54), respectively, in predicting vitamin B12 deficiency. A ≥ six (6) years duration of metformin therapy presented with a sensitivity, specificity, and AUC of 70.4%, 62.9%, and 0.66 (95% CI, 0.57-0.75), respectively, in predicting vitamin B12 deficiency.

Conclusion: Vitamin B12 deficiency is high among T2DM patients on metformin therapy in Ghana. There is the need for regular monitoring of vitamin B12 levels especially in T2DM patients on metformin daily dose of ≥ 1500 mg for duration of therapy ≥ 6 years.

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Type 2 diabetes mellitus; metformin; vitamin B_{12} deficiency; methylmalonic acid

1. Introduction

Diabetes mellitus (DM) is a pervasive chronic metabolic disorder and accounts for up to 90% of all diagnosed diabetes [1,2]. Type 2 diabetes mellitus (T2DM) is the most common form of DM, accounting for approximately 90% of DM cases [3]. In Ghana, 6.3% and 6.4% have been quoted as crude and age-adjusted prevalence of T2DM [4,5]. T2DM is associated with several detrimental microvascular and macrovascular complications [6]. As such, effective management of the disease is crucial.

The American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD) recommend metformin as the first therapeutic choice for T2DM management with concurrent lifestyle modifications. Reports indicated that metformin improves peripheral insulin sensitivity and reduces the risk of cardiovascular mortality in T2DM [7,8] in addition to its beneficial effects on weight loss and vascular protection [9]. Nonetheless, as with most medications, the use of metformin present with some side effects. Though most of these side effects are mild, reports indicate that metformin use is associated with the diminution of the terminal iliac uptake of vitamin B_{12} [10,11]. Other studies also report that long-term use of metformin results in vitamin B_{12} malabsorption, with a concomitant reduction in serum vitamin B_{12} levels [11–13]. Metformin-induced Vitamin B_{12} deficiency has been reported to be duration of therapy- and dose-dependent [12].

Metformin-induced vitamin B_{12} deficiency has also been associated with neuropathy. The neuropathy associated with vitamin B_{12} deficiency ranges from paresthesia and attenuated peripheral sensation in response to changes in mental status and proprioception [7] which overlap with diabetic neuropathy. The progression of vitamin B_{12} deficiency-induced neurologic damage can,

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however, be abated through early detection and vitamin B_{12} therapy [14]. Nonetheless, if peripheral neuropathy due to deficiency of vitamin B_{12} is misconstrued as diabetic peripheral neuropathy, permanent neurological damage may occur [15].

Despite the growing evidence of metformin-induced vitamin B_{12} deficiency, the prevalence and association between metformin use and vitamin B_{12} deficiency in the Ghanaian population is yet to be elucidated. Furthermore, there are no guidelines to address how often Ghanaian T2DM patients on metformin should be screened for the risk of vitamin B_{12} deficiency which would inform appropriate prescription of vitamin B_{12} supplements probably due to limited studies on the subject matter.

Diagnosis of vitamin B_{12} deficiency can, however, be difficult. Reports indicate that high serum vitamin B_{12} levels can be accompanied by signs of deficiency, and functional deficiency from tissue uptake defects and action of vitamin B_{12} at the cellular level have been implicated in this association [16,17]. Thus, functional vitamin B_{12} deficiency can occur regardless elevated serum B_{12} levels [17]. As such, a more sensitive screening method is warranted. One such method exploits metabolites that accumulate due to vitamin B_{12} deficiency. Vitamin B_{12} is involved in the conversion of methylmalonic acid (MMA) to succinyl-CoA as well as homocysteine (HC) to methionine in combination with folic acid [18]. Serum vitamin B_{12}, in combination with MMA levels, have been demonstrated to be effective in classifying vitamin B_{12} deficiency compared to serum vitamin B_{12} alone [16,19].

This study, therefore, employed both serum vitamin B_{12} and MMA levels to estimate the prevalence of vitamin B_{12} deficiency and identify risk factors associated with vitamin B_{12} deficiency among Ghanaian T2DM patients.

2. Materials and methods

2.1. Study design / setting

This cross-sectional study was conducted at the Diabetic clinic of the Effia Nkwanta Regional Hospital, Takoradi, Ghana. Effia Nkwanta Hospital is a referral center for peripheral health facilities in the Western Region. The diabetic clinic offers general and specialized care for diabetes patients.

2.2. Ethical consideration

The study protocol was reviewed and approved by the Committee for Human Research, Publications and Ethics of SMS/KNUST/KATH and institutional approval was obtained from Effia Nkwanta Regional Hospital. Written informed consent was obtained from participants after the objectives and benefits of the study were explained to them. Participation was entirely voluntary and strict confidentiality of participants' information was maintained throughout the study.

2.3. Study population

The sample size for this study was calculated using the Raosoft sample size calculator [20]. At 95% confidence level, 7% margin of error, and a response distribution of 50%, the minimum sample size for the study was 180 T2DM patients. In an effort to enhance the statistical power of the study, a total of 250 T2DM patients were targeted for the study. However, upon excluding subjects who did not fulfill the inclusion criteria, a total of 196 participants were enrolled in the study. Non-probability sampling technique was employed to recruit 196 consecutive consenting participants who were already diagnosed of T2DM and on treatment. Subjects recruitment occurred during patients' routine clinic visit. Since T2DM is considered a disease of the aged, patients aged 35–65 years old, who had been on metformin, with minimum daily dose of 500 mg, for at least 6 months were included. T2DM patients who were pregnant/lactating, alcoholics or drug abuse, known cases of malabsorption (gastrointestinal surgery, inflammatory bowel diseases and gluten allergy), chronic kidney disease, pernicious anemia, thalassaemia, sickle cell anemia, consumption of vitamin B_{12} or calcium supplementation during the last 3 months and receiving antibiotics or any medications known to influence gastrointestinal motility were excluded from the study. All relevant clinical data were retrieved from the hospital's archive. Participants' selection protocol is shown in Figure 1.

2.4. Questionnaire administration

Validated questionnaire was used for data collection. The questionnaire gathered information on demographic characteristics, anthropometric variables, medications (type, dose, and duration) and symptoms of neuropathy. Patients information from the hospital's archive were cross-referenced to confirm responses such as medication dose, duration of use, and duration of diabetes.

2.5. Blood pressure and anthropometric measurements

Blood pressure was measured from the left upper arm using a mercury sphygmomanometer and a stethoscope by a trained nurse at the diabetic clinic. Participants were asked to rest for at least 5 min before measurement. The average of the two readings taken 5 min apart was recorded as the blood pressure measurement.

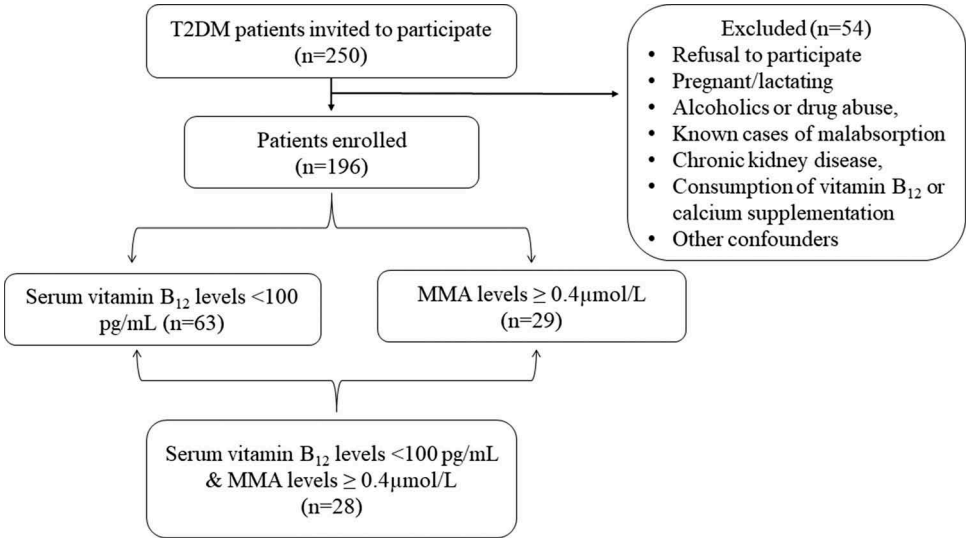

Figure 1. Flowchart of the protocol for the selection of subject.

Height was measured without wearing shoes using a wall-mounted ruler, to the nearest 0.1 m. Waist and hip circumferences were measured to nearest centimeter using a measuring tape. Waist to hip ratio (WHR) = WC (m)/HC (m), waist to height ratio and (WHtR) = WC (m)/height (m) were calculated [21]. Total body fat, percentage visceral fat (VF) and skeletal muscle were also estimated using the Omron Body Composition Monitor (Omron Corporation, Japan). Body mass index (BMI) was calculated using the equation; [BMI (kg/m^2) = weight/height^2] [22].

2.6. Sample collection, preparation and biochemical assays

Five millimeters (5 ml) of venous blood sample was collected after an overnight fast. One (1) milliliter of blood was dispensed into tubes containing fluoride oxalate, another 1 ml was dispensed into tubes containing EDTA, and the remaining 3 ml of blood was dispensed into gel separator tubes. The sample in the EDTA tube was used for estimation of hemoglobin (Hb) and glycated hemoglobin (HbA1c) while the sample in the fluoride oxalate was used for glucose estimation (FPS). The gel separator tubes were placed in a centrifuge and span at 3000 rpm for 5 min to obtain the serum. Hb, HbA1c, and FPS were measured immediately and the serum for the measurement of other biochemical variables were stored at −20°C until analysis. FPS, HbA1c, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and calcium were estimated using an automated chemistry analyzer (Selectra Pro S System, Elitech Group, France). The estimation of serum vitamin B_{12} was based on solid-phase Enzyme Linked Immunosorbent Assay (ELISA) method (standardized with an intra-assay %CVs = 4.4–5.6% and inter-assay %CVs = 6.6–7.9%) (MyBioSource, Inc. San Diego, CA, USA) according to the manufacturer's instructions. Briefly, 100 µL of standards, samples and controls were pipetted into appropriate microtitre wells followed by 100 µL of enzyme conjugate reagent, mixed thoroughly, covered with an adhesive strip, and incubated at 37°C for 60 min. The incubation mixture was aspirated from the wells followed by five washes with the wash solution (400 µL). Residual water droplets were removed by striking the wells onto absorbent paper. A 50 µL of Substrate A and 50 µL of Substrate B were pipetted into each well, mixed gently and incubated at 37°C for 15 min.

The estimation of serum methylmalonic acid was based on the competitive binding enzyme immunoassay technique (standardized with an intra-assay %CVs <6.1% and inter-assay %CVs <10.2%) (MyBioSource, Inc. San Diego, CA, USA) according to the manufacturer's instructions. Briefly, 50 µL of standards, controls, and samples were pipetted into appropriate microtitre wells which were pre-coated with an antibody specific to MMA. Fifty microliters (50 µL) of Detection A working solution was added to each well, mixed thoroughly, covered with an adhesive strip, and incubated at 37°C for 60 min. The incubation mixture was aspirated from the wells followed by five washes with the wash solution (400 µL). Residual water droplets were removed by striking the wells onto absorbent paper. A 100 µL of Detection A working solution was pipetted into each well, mixed gently and incubated at 37°C for 45 min.

Both reactions were stopped by adding 50 µL of Stop Solution to each well and gently mixed for 30 s. The absorbance of the final colored product was measured spectrophotometrically at 450 nm using Thermo Electron Multiskan EX plate reader (Shanghai, China). The mean absorbance value (OD_{450}) for each set of reference standards, controls

and samples were calculated. The calculated mean OD_{450} obtained for each reference standard were used to construct a standard curve and the concentrations of samples and controls determined from the standard curve. Vitamin B_{12} deficiency was defined by B_{12} levels <100 pg/mL based on measurement of serum vitamin B_{12} and ≥0.4 µmol/L based on MMA levels [23,24]. Daily calibration and maintenance of analyzer was performed according to the manufacturer's instructions as previously described [25]. Quality control (QC) was assessed using quality control materials provided by the manufacturer.

2.7. Statistical analysis

Categorical data were presented as frequencies and percentages, and Chi-square and Fisher exact test were used to test for significance of associations where applicable. Parametric and non-parametric continuous data were presented as means ± SD and medians (IQR), respectively, and Independent t-test and Mann Whitney U test were used to test significance of associations where applicable. Multivariate logistic regression model was used to identify risk factors associated with vitamin B_{12} deficiency after adjusting for age, sex, overweight, anemia, insulin and sulfonylurea use, DM and medication duration. The covariates selected for the multivariate regression were known or surmised factors that could influence vitamin B_{12} status. The receiver operating characteristic (ROC) curve was used to determine the cutoff value of metformin dose and duration of metformin therapy associated with vitamin B_{12} deficiency. The optimum cutoff value was determined based on the highest Youden index [J = (sensitivity + specificity) – 1]. A ρ value <0.05 was considered statistically significant. Data processing was done using Microsoft Excel 2016. Statistical analysis and graphical presentation were performed using the R Language for Statistical Computing version 3.5.2 (R Core Team, Vienna, Austria) [26].

3. Results

A total of 196 T2DM patients with mean age of 50.4 (±6.8) years were included in this study. The median duration of T2DM and metformin therapy was 6.0 (3.0–10.0) years and 5.0 (3.0–8.0) years, respectively. There were more females (69.9%) than males (30.1%). The prevalence of anemia based on hemoglobin level was 45.9% and the average vitamin B_{12} and MMA levels among the entire study population were 205.4 pg/ml and 0.07 µmol/L, respectively (Table 1).

The prevalence of vitamin B_{12} deficiency based on serum vitamin B_{12} (sVB_{12}), MMA, and the combination of both methods (sVB_{12}+ MMA) were 63/196 (32.1%), 29/196 (14.8%), and 28/196 (14.3%), respectively (Figure 2).

Table 1. Baseline characteristics of entire study subjects.

Variables	Mean ± SD
Age (years)	50.4 ± 6.8
Sex[a]	
Male	59 (30.1)
Female	137 (69.9)
Hb (g/dL)	12.2 ± 1.2
Anemic status[a]	90 (45.9)
Biochemical Parameters	
FPG (mmol/L)[b]	8.2 (6.5–11.8)
HBA1c (%)	7.1 ± 1.8
TC (mmol/L)	5.2 ± 1.3
TG (mmol/L)	1.7 ± 0.6
HDL-C (mmol/L)	1.2 ± 0.3
LDL-C(mmol/L)	3.7 ± 1.2
Calcium (mmol/L)	2.4 ± 0.2
Vitamin B_{12} (pg/ml)[b]	202.1(132.1–253.8)
MMA (µmol/L)[b]	0.07(0.01–0.2)
Hemodynamic indices	
SBP(mmHg)	137.8 ± 18.7
DBP(mmHg)	83.6 ± 10.6
Anthropometric Indices	
BMI (kg/m^2)	28.6 ± 5.6
WHR	0.9 ± 0.1
WHtR	0.6 ± 0.1
Visceral fat (%)[b]	9.5(6.0–14.0)
Body fat (%)[b]	30.3(24.9–38.0)
Skeletal muscle (%)	28.1 ± 4.7
Duration of DM (years)[b]	6.0(3.0–10.0)
Duration of metformin therapy (years)[b]	5.0(3.0–8.0)

Parametric data are presented as mean ± standard deviation, while non-parametric data presented as median (interquartile range), Hb: Hemoglobin level, FPG: Fasting Plasma Glucose, HBA1c: Glycated hemoglobin, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High Density Lipoprotein Cholesterol, LDL-C Low Density Lipoprotein, MMA: Methylmalonic Acid, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body mass index, WHR: Waist–hip ratio, WHtR: waist-to-height ratio. Anemia was defined as hemoglobin level <12 g/dl for females and 13 g/dl for males.
[a]Presented as n (%).
‡[b]Presented as median (interquartile range).

There were no statistically significant differences between the demographic, anthropometric, biochemical parameters and vitamin B_{12} status (Table 2).

Vitamin B_{12} deficient subjects presented with significantly longer duration of T2DM [10.0 (7.0–11.8) vs 5.0 (3.0–9.0); p = 0.007] and duration of metformin use [6.0 (4.0–11.0) vs 4.0 (3.0–7.0); p < 0.0001] compared to non-deficient subjects. Subjects with vitamin B_{12} deficiency significantly used higher doses of metformin (>2000 g/day) compared to non-deficient subjects (Table 3).

In the multivariate logistic regression, after adjusting for possible confounding variables, longer duration of metformin use [5–9 years; aOR = 2.83, 95% CI (1.03–7.81), p = 0.045 and ≥10 years; aOR = 4.17, 95% CI (1.41–12.33), p = 0.010], higher daily dose of metformin [1000–2000 mg/day; aOR = 1.34, 95% CI (0.25–2.74), p = 0.038 and >2000 mg/day; aOR = 1.13, 95% CI (0.39–2.97), p = 0.047], and very high body fat [aOR = 2.98, 95% CI (1.47–6.05), p = 0.020] were significantly associated with increased odds of vitamin B_{12} deficiency (Table 4).

For daily dose of metformin, a cutoff value of 1500 mg/day presented with a sensitivity, specificity, and AUC of 71.4%, 40.1%, and 0.54 (95% CI, 0.53–0.54), respectively, in predicting vitamin B_{12} deficiency. Additionally, ≥ six (6) years duration of

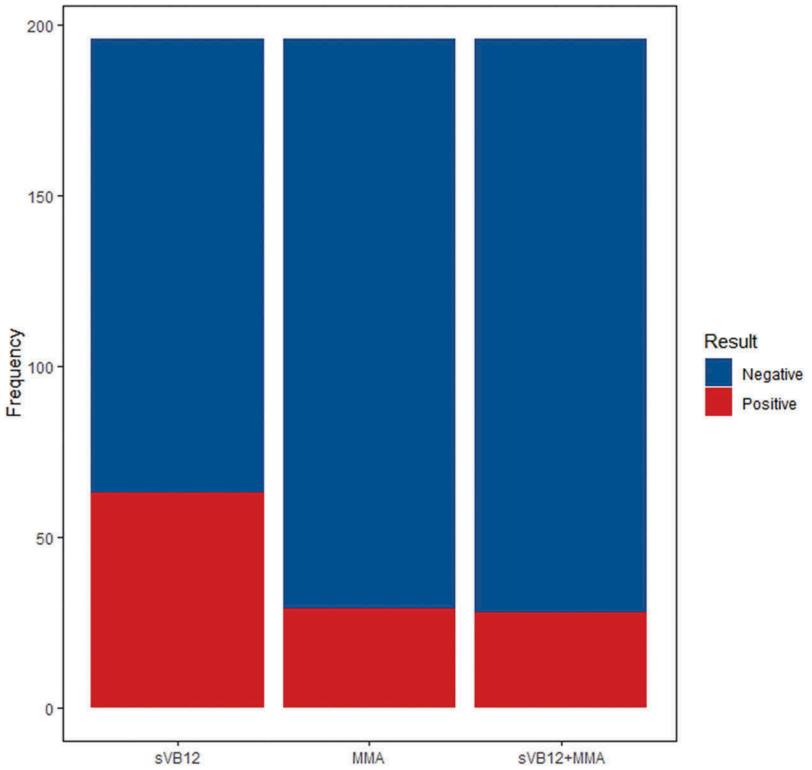

Figure 2. Prevalence of vitamin B_{12} deficiency among the entire study population.

Table 2. Demographic, anthropometric and biochemical parameters of the study population stratified by vitamin B_{12} status.

	Vitamin B_{12} status*		
Variables	Deficient (n = 28)	Non-deficient (n = 168)	p-value
Age (years)	51.6 ± 6.5	50.3 ± 6.7	0.328
Sex[a]			0.278
Male	11(18.3)	49(81.7)	
Female	17(12.5)	119(87.5)	
Hb (g/dL)	12.1 ± 1.5	12.3 ± 1.3	0.410
Anemic status[a]			0.684
Anemic	14(15.7)	75(84.3)	
Non-anemic	14(13.2)	92(86.8)	
FPG (mmol/L)[b]	8.7(5.5–12.5)	8.2(6.6–11.6)	0.219
HBA1c (%)	7.2 ± 1.3	7.1 ± 1.9	0.997
TC(mmol/L)	4.9 ± 1.0	5.3 ± 1.3	0.164
TG(mmol/L)	1.6 ± 0.5	1.8 ± 0.6	0.296
HDL-C(mmol/L)	1.1 ± 0.3	1.2 ± 0.3	0.212
LDL-C(mmol/L)	3.5 ± 1.0	3.7 ± 1.2	0.267
Calcium(mg/dL)	2.3 ± 0.5	2.4 ± 0.2	0.066
BMI (kg/m^2)	29.9 ± 6.8	28.4 ± 5.4	0.219
WHR	0.9 ± 0.1	0.9 ± 0.1	0.421
WHtR	0.6 ± 0.1	0.6 ± 0.1	0.238
Skeletal muscle (%)	27.2 ± 3.9	28.2 ± 4.8	0.210

Hb: Hemoglobin level, FPG: Fasting Plasma Glucose, HBA1c: Glycated Hemoglobin, TC: Total Cholesterol, TG: Triglyceride, HDL-C: High-Density Lipoprotein Cholesterol, LDL-C Low-Density Lipoprotein. BMI: Body mass index, WHR: Waist-to-hip ratio, WHtR: Waist-to-height ratio. Data are presented as mean ± SD unless otherwise specified.
*Based on the prevalence from the combination of serum vitamin B_{12} and MMA.
[a]Presented as frequency (%)
[b]Presented as median (interquartile range)

metformin therapy presented with a sensitivity, specificity, and AUC of 70.4%, 62.9%, and 0.66 (95% CI, 0.57–0.75), respectively, in predicting vitamin B_{12} deficiency (Figure 3).

4. Discussion

This study reports a high prevalence of vitamin B_{12} deficiency among Ghanaian T2DM on metformin. The prevalence of vitamin B_{12} deficiency based on serum vitamin B_{12}, MMA, and the combination of both methods was 32.1%, 14.8%, and 14.3%, respectively. Vitamin B_{12} deficient subjects presented with significantly longer duration of T2DM, duration of metformin use and used higher metformin doses compared to non-deficient subjects. Using multivariate logistic regression models, after controlling for multiple potential covariates observed in other

Table 3. Comparison of medication information and symptoms of study participants stratified by vitamin B_{12} status.

	Vitamin B_{12} status		
Variables	Deficient (n = 28)	Non-deficient (n = 168)	p-value
Duration of DM (years)[a]	10.0(7.0–11.8)	5.0(3.0–9.0)	**0.007**
Duration of met use (years)[a]	6.0(4.0–11.0)	4.0(3.0–7.0)	**<0.0001**
Metformin daily doses (mg/day)			**<0.0001**
<1000	4 (14.3)	99(58.9)	
1000–2000	6(21.4)	27(16.1)	
>2000	18(64.3)	42(25.0)	
Metformin +other drugs (Yes, %)	22(78.6)	107(63.7)	0.138
Sign of weakness (Yes, %)	7(25.0)	60(35.7)	0.291
Constipation (Yes, %)	4(14.3)	11(6.5)	0.239
Diarrhea(Yes, %)	1(3.6)	11(6.5)	1.000
Loss of appetite (Yes, %)	0(0.0)	12(7.1)	0.221
Sign of nerve problems (Yes, %)	16(57.1)	104(61.9)	0.607
Gait problem (Yes, %)	5(17.9)	19(11.3)	0.352
Blurred Vision (Yes, %)	16(57.1)	64(38.1)	0.066
Sign of memory loss (Yes, %)	3(10.7)	50(29.8)	0.087
Weight loss (Yes, %)	6(21.4)	64(38.1)	0.085

[a]Presented as median (interquartile range).

Table 4. Possible risk factors for vitamin B_{12} deficiency among the study population.

	Vitamin B_{12} deficiency	
Variables	aOR (95% CI)	p-value
Age (per year)	4.12(0.77–21.99)	0.623
Gender (male)	1.5(0.82–2.94)	0.180
BMI (kg/m^2)	1.02(0.96–1.08)	0.464
WHtR	5.68(0.17–189.2)	0.331
WHR	101.9(0.48–2160.9)	0.742
Body Fat		
Normal	1	
Low	0.82(0.33–2.06)	0.295
High (+)	2.06(0.53–7.97)	0.675
Very High (++)	2.98(1.47–6.05)	**0.020**
Anemia	4.12(0.77–21.99)	0.098
Medication used		
Metformin only	1	
Metformin+ Insulin	0.84(0.37–1.93)	0.608
Metformin+ Sulfonylurea	0.73(0.32–1.61)	0.434
Duration of metformin use (years)		
<5	1	
5–9	2.83(1.03–7.81)	**0.045**
≥10	4.17(1.41–12.33)	**0.010**
Metformin daily dose (mg)		
<1000	1	
1000–2000	1.34(0.25–2.74)	**0.038**
>2000	1.13(0.39–2.97)	**0.047**
Duration of DM (years)		
<5	1	
5–9	1.40(0.41–4.83)	0.591
≥10	4.02(2.06–17.57)	0.072

BMI: Body mass index, WHtR: Waist-to-height ratio, WHR: Waist-to-hip ratio, DM: Diabetes mellitus. Multivariate logistic regression model was used to identify risk factors associated with vitamin B_{12} deficiency after adjusting for age, sex, overweight, anemia, insulin and sulfonylurea use, DM and medication duration. A ρ value < 0.05 was considered statistically significant (*p* values of significant variables in bold print).

studies, we found longer duration of metformin use (≥5 years), higher daily dose of metformin (≥1000 mg/day) and very high body fat to be significantly associated with increased risk of vitamin B_{12} deficiency. In determining the optimal cutoff value for daily dose of metformin and duration of metformin therapy in predicting vitamin B_{12} deficiency, we employed the ROC curve analysis. We found metformin dose of 1500 mg/day and ≥six (6) years duration of metformin therapy to be predictive of vitamin B_{12} deficiency among the study population.

Varying prevalence rates of vitamin B_{12} deficiency among T2DM patients on metformin have been reported in diverse populations. Report from an early clinical observational study by Tomkin et al. [27] among Irish diabetic patients on long-term metformin therapy with concomitant dietary management indicated a prevalence of 30% for vitamin B_{12} malabsorption. DeFronzo et al. also reported a prevalence of 29% among American T2DM patients using metformin [28]. Another study by Sparre Hermann et al. reported a 26.7% prevalence of vitamin B_{12} deficiency in metformin-treated T2DM patients in Sweden [29]. Additionally, a study by Damião et al. [30] and Pflipsen et al. [23] found a 22.5% and 22% prevalence of B_{12} deficiency among Brazilian and American T2DM patients using metformin. Wulffele

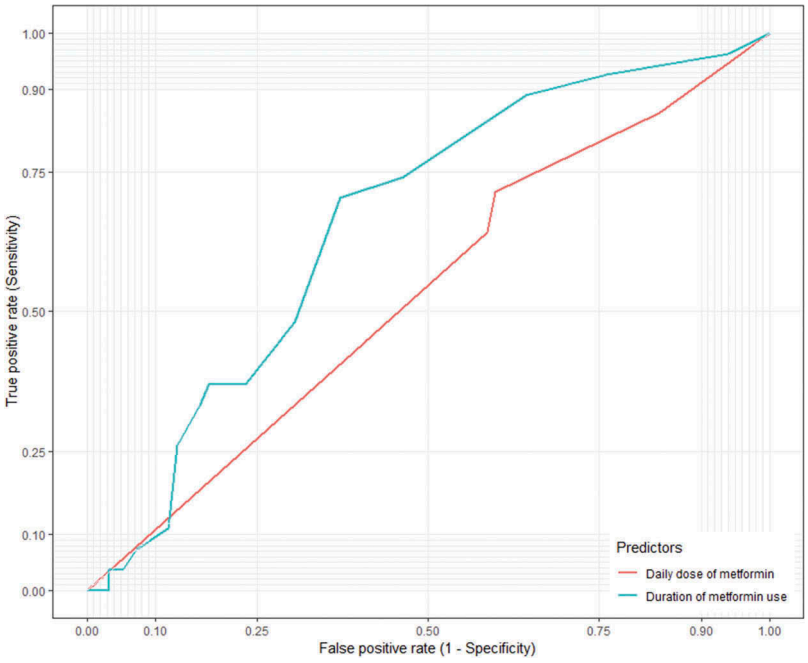

Figure 3. Receiver operating characteristic (ROC) curve analysis for the duration and daily dose of metformin in relation to vitamin B_{12} deficiency.

et al. also reported a vitamin B_{12} deficiency prevalence of about 14% among T2DM patients treated with metformin in the Netherlands [31]. Another study by Ko et al. reported a 9.5% prevalence of vitamin B_{12} deficiency in metformin-treated T2DM patients in Korea [32]. Furthermore, a study by Reinstatler et al. among adults ≥50 years of age from NHANES 1999–2006 found that biochemical vitamin B_{12} deficiency was present in 5.8% of T2DM patients using metformin and 2.4% among those not using metformin [33]. Compared to the recent studies, the alarmingly high prevalence of vitamin B_{12} deficiency given the conservative cutoff points utilized in this study is striking. This underscores the need for apt guidelines to address how often T2DM patients on metformin should be screened for the risk of vitamin B_{12} deficiency which would inform appropriate prescription of vitamin B_{12} supplements. That aside, the discrepancies in prevalence rates may be attributed to disparities in the cutoff values for B_{12} deficiency, influence of geographical variations and dietary characteristics. Though the mechanisms underpinning metformin-induced vitamin B_{12} deficiency have not been fully elucidated, bacterial overgrowth in the small intestine due to DM, changes in the bacterial flora, alterations in small bowel motility, the inactivation of vitamin B_{12} absorption, and the effect of calcium on cell membranes have been proposed [10,12,27,31].

Clinically, vitamin B_{12} deficiency has been associated with macrocytic anemia, neuropathy, and mental changes [7,15,34,35]. Thus, though anemia observed in this study may have multifactorial causes, vitamin B_{12} deficiency-associated macrocytic anemia may be the most probable [34,35]. Importantly also, the neurologic damage can present as peripheral neuropathy and may be misconstrued for diabetic neuropathy in patients on metformin therapy [7,15]. Thus, since vitamin B_{12}-associated neuropathy is reversible, prompt detection and treatment of vitamin B_{12} deficiency among T2DM patients on metformin is crucial to avert permanent neurological damage. Nonetheless, it is worthy of note that we did not observe significant association between the manifestations of neuropathy and anemia with vitamin B_{12} deficiency. A possible reason for this finding may be due to the direct neuroprotective effect of metformin through its glucose-lowering effect and antihyperglycemic-independent, direct anti-neuropathic impact on neurons including inhibition of oxidative stress-related apoptotic cell death [36,37]. Studies by Ahmed et al. in South Africa and Russo et al. in Italy [38,39] also found no significant difference in the presence of neuropathy between subjects with normal vitamin B_{12} levels and those with vitamin B_{12} deficiency. Additionally, though classical B_{12} deficiency is associated with clinical symptoms such as anemia and peripheral neuropathy, these symptoms are usually absent in those with only biochemical vitamin B_{12} deficiency [33]. This may be the reason for the non-significant association between the vitamin B_{12} deficiency and anemia observed in this study.

The association of duration of metformin use and vitamin B_{12} deficiency found in this study is expected as similar findings have been reported by previous studies. A study by Bauman et al. found that 12 out of 14 T2DM patients presented with reduced serum total vitamin B_{12} levels after 3 months of metformin therapy [10]. A study by Ting et al. also found increased duration of metformin use to be associated with more than two-fold increased risk of developing

vitamin B_{12} deficiency [12]. Another study by De Jager et al. found that the absolute risk of vitamin B_{12} deficiency after a period of 4 years was 7.2% higher in T2DM patients on the metformin [40]. A similar finding has also been reported by Wile and Toth [13], Wulffelé et al. [31], and Andrès et al. [41]. Despite the coherence with previous studies, metformin-induced vitamin B_{12} deficiency in this study appeared to be duration of use- and dose-dependent similar to the findings of Beulens et al. [42]. Ko et al. [32] found that T2DM patients who had taken metformin >1,000 mg/day were approximately 10 times as likely to have vitamin B_{12} deficiency as patients taking metformin ≤1,000 mg/day which is in harmony with this present study. However, Ko et al. also found that this dose (>1,000 mg/day) was associated with vitamin B_{12} deficiency after ≥45.5 months (≈12 years) of therapy which is a longer period compared to the cutoff predictive of vitamin B_{12} deficiency in this study (≥6 years). The longer duration of metformin use may be due to dissimilarities in the cutoff values of B_{12} used. Ko et al. defined vitamin B_{12} deficiency as serum levels ≤300 pg/mL while we defined vitamin B_{12} deficiency as serum levels of both vitamin B_{12} < 100 pg/mL and MMA ≥ 0.4μmol/L. The higher cutoff level in their study may have allowed for the inclusion of more vitamin B_{12} deficient subjects with longer duration of metformin therapy, which may have been missed in this study, consequently influencing risk associations. Additionally, the interaction of duration of T2DM and duration of metformin therapy may be partly involved.

Also of note is the association between vitamin B_{12} deficiency and very high body fat. A study by Baltaci et al. [43] reported that obesity based on bioelectric fat analysis (total body fat %) was significantly associated with vitamin B_{12} deficiency which is in harmony with our study finding. Also consistent with our finding is a study by Pinhas-Hamiel et al. [44] who reported a greater than fourfold increased risk of reduced vitamin B_{12} status in Israeli obese compared to normal-weight children and adolescents. Though the mechanisms underpinning the influence of obesity in vitamin B_{12} deficiency is still under investigation, proposed mechanisms include obesity-induced B_{12} malabsorption, poor dietary content, repeated short-term restrictive diets and increased requirements frequently seen in obese people [44–46]. Clinically, however, metformin is frequently prescribed at higher doses and for longer duration among obese T2DM patients. Thus, our finding suggests that, a more frequent assessment of vitamin B_{12} status for obese T2DM patients using metformin may have immense positive influence in abating the high prevalence rate of metformin-induced vitamin B_{12} deficiency.

Despite interpreting our results with caution, some limitation of the study should be acknowledged. One of which is the cross-sectional design of this study which precluded the establishment of the causal relation between metformin and vitamin B_{12} deficiency. Thus, case-control or longitudinal studies are required to prove any causality in this association. The small sample size is also a limiting factor. Therefore, the findings may not be generalizable to the general population. The issue of external validity is also of concern because the study was conducted in a single urban center and significant disparities in prevalence rates may exist from in different the regions. A follow up using a larger sample size is, thus, warranted.

5. Conclusion

It was evident from this study that vitamin B_{12} deficiency is frequent in Ghanaian T2DM patients on metformin. The study also confirms findings from other countries that daily dose and duration of metformin are risk factors for the development vitamin B_{12} deficiency. However, these findings should not be considered as a basis for discontinuing of the use of metformin in the treatment of T2DM because metformin has been proven to produce a better glycemic control in T2DM. Rather, there is the need for regular monitoring of vitamin B_{12} levels especially in patients on ≥1500 mg daily dose of metformin for a duration of six or more years.

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article.

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