Original Article

Serum vitamin B12 status of patients with type 2 diabetes mellitus on metformin: A single-center cross-sectional study from Bangladesh

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Abstract

Background: Metformin use is a known cause of B12 deficiency in patients with type 2 DM (T2DM). Diabetic peripheral neuropathy (DPN) often has clinically indistinguishable clinical features of B12 deficiency-induced peripheral neuropathy (PN). **Objective:** The present study aims to assess serum vitamin B12 levels in patients with T2DM on metformin. **Subjects and Methods:** This cross-sectional study was conducted at a specialized endocrine outpatient clinic in Cumilla, Bangladesh, over six months from January 2020 to June 2020. Non-pregnant adults (≥18 years age) receiving metformin for T2DM for at least six months were evaluated for PN and assessed for serum B12 levels. **Results:** Among 90 subjects evaluated, 28 (31.1%) had B12 deficiency and 6 (6.7%) had borderline B12 deficiency; 56 (62.2%) had normal B12 levels. Study subjects with subnormal B12 used metformin for a longer duration [8.5 (7.0-14.0) vs. 5.0 (2.25-10.0) years, median (IQR), $P = 0.006$], gram-years of metformin use was higher in them [12.0 (7.9-14.0) vs. 5.75 (2.0-13.6) years, median (IQR), $P = 0.005$] and they had a higher mean corpuscular volume [85.9±7.2 vs. 82.4±6.4 fL, mean±SD, $P = 0.020$] compared to those having normal B12 levels. Serum B12 levels had a strong negative correlation with duration of metformin use and gram-years of metformin use. B12 status did not influence the presence and severity of PN. **Conclusions:** A considerable number of patients with T2DM have subnormal B12 levels. Periodic screening for serum vitamin B12 level may be of clinical benefit in such patients.

Keywords: Metformin, peripheral neuropathy, vitamin B12, type 2 diabetes

Introduction

Diabetes mellitus (DM) affects about 463 million adults worldwide currently. Type 2 DM (T2DM) is the most common type of diabetes, accounting for around 90% of all diabetes.[1] Metformin is the first-line drug combined with lifestyle modifications for the treatment of T2DM; other agents, including insulin, should be added to metformin unless metformin is contraindicated or not tolerated by the patient.[2,3] Metformin is a time-tested drug in T2DM management that is effective, has a relatively safe adverse effect profile, is inexpensive, and may reduce the risk of cardiovascular events and death.[2,3] Metformin use is a known cause of vitamin B12 deficiency in patients with T2DM, which was first described by Berchtold *et al.* in 1969 and has been demonstrated by many researchers since then.[4-9] The reported prevalence of metformin-related vitamin B12 deficiency is widely variable (5--40%) in the available literature.[8,10]

Clinical pictures of peripheral neuropathy caused by DM and vitamin B12 deficiency may be overlapping.[11,12] The high

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prevalence of B12 deficiency in DM makes it likely that at least a portion of these patients' peripheral neuropathy cases may be attributable to B12 deficiency.[12,13] Therefore, measurement of serum B12 level in patients with T2DM may help explore concurrent B12 deficiency, which can be treated very easily. Most of the patients with T2DM in Bangladesh are managed by general physicians (GPs), and metformin is a commonly prescribed drug in the country for managing the condition.[14] Although all physicians, including the GPs, should have proper knowledge of long-term metformin-related side effects, including B12 deficiency, data on B12 status in metformin-treated patients with T2DM is scarce in Bangladesh. The present study aims to measure serum vitamin B12 levels in patients with T2DM on metformin.

Subjects and Methods

This cross-sectional study was conducted at a specialized endocrine outpatient clinic in Cumilla city of Bangladesh from January 2020 to June 2020. Non-pregnant adults (≥18 years age) receiving metformin for the treatment for T2DM for at least 6 months were included in this study. Vegetarians, subjects having any comorbid conditions that may interfere with vitamin B12 level (e.g., pernicious anemia, malabsorption, gastrointestinal surgery), those with thyroid disorders, advanced hepatic and renal disease, those with regular (3 months or longer duration) use of acid suppressants (i.e., proton pump inhibitors or H_2 receptor blockers), chronic alcohol abuse, getting supplementation of B12 or any B12 containing multivitamin, either by the oral or parenteral route, concomitant drugs use that affect B12 level (e.g., corticosteroids, phenytoin, dihydrofolate reductase inhibitors), were excluded. Samples were collected using a convenient sampling technique. Informed written consent was taken from each study subject after a complete explanation of the study's steps and purpose. The participants were interviewed and examined for demographic and relevant clinical information. Assessment of peripheral neuropathy was done by using Toronto Clinical Neuropathy Scoring System (TCNS), and the presence of peripheral neuropathy will be interpreted as no neuropathy: 0--5 points, mild neuropathy: 6--8 points, moderate neuropathy: 9--11 points, and severe neuropathy: 12+ points.[15] Data were collected using a pretested case record form. Random venous blood samples were collected, and assessments of complete blood count (CBC), serum creatinine, HbA1c, and serum vitamin B12 were done. Serum vitamin B12 was measured on the same day of blood sample collection (preferably within 08 hours) by Chemiluminescent Microparticle Immunoassay (CMIA) technology. Serum vitamin B12 level ≤200 pg/mL was labeled as a deficiency, >200 to ≤300 pg/mL as a borderline deficiency, and >300 pg/mL was labeled normal.[6] Anemia was defined as hemoglobin concentration <13.0 gm/dL in men and <12.0 gm/dL in women.[16] Ethical approval was taken on 20.06.2017.

Statistical analysis

The Statistical Product and Service Solutions (SPSS) for Windows, version 26.0 software (IBM Corp. Released 2019, Armonk, NY) was used to analyze data. The measurable variables with normal distribution were presented as mean ± standard deviation (SD), and those not following normal distribution were presented as median; categorical variables were presented as frequencies (percentages). Student's *t*-test, Chi-square test, and Mann--Whitney U test were performed to compare the variables between different groups. Pearson's correlation test was used to observe the correlation of B12 level with other variables. *P* value ≤ 0.05 was considered to be statistically significant.

Results

Among 90 subjects evaluated, 28 (31.1%) had B12 deficiency and 6 (6.7%) had borderline B12 deficiency; 56 (62.2%) had normal B12 levels [Figure 1].

The clinical, metabolic, and other biochemical characteristics of the study subjects having normal and subnormal (borderline deficiency and deficiency) B12 are given in Table 1. Study subjects with subnormal B12 used metformin for a longer duration, gram-years of metformin use was higher in them, and they had a higher mean corpuscular volume (MCV) than those having normal B12 levels.

In Pearson correlation analysis, serum B12 levels had a strong negative correlation with duration of metformin use and gram-years of metformin use [Table 2].

Discussion

In the present study, out of 90 patients with T2DM on metformin, 37.8% had subnormal (6.7% deficiency and 31.1% borderline) serum vitamin B12 levels. Study subjects with subnormal B12 used metformin for a longer duration, gram-years of metformin use was higher in them, and they had a higher mean corpuscular volume (MCV) than those having normal B12 levels. Serum B12 levels had a strong negative correlation with duration of metformin use and gram-years of metformin use.

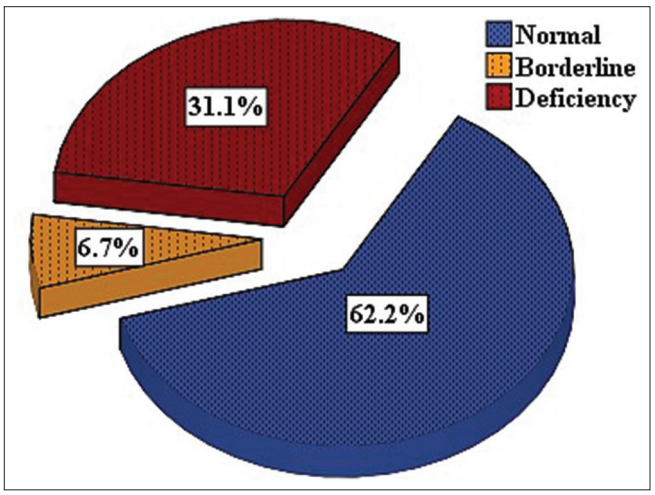

Figure 1: Vitamin B12 status of the study subjects

Table 1: Comparison of the clinical, metabolic, and other biochemical variables of the subjects with normal B12 and subnormal (borderline and deficiency) B12

Variables	Subgroups	Mean±SD or median (IQR) or n (%)			*P*
		All Subjects (*n*=90)	Normal B12 (*n*=56)	Subnormal B12 (*n*=34)	
Age (years)		48.7±9.5	49.2±9.3	47.9±9.8	0.526
Gender	Male	37 (41.1)	23 (41.1)	14 (41.2)	1.000
	Female	53 (58.9)	33 (58.9)	20 (58.8)	
Residence	Urban	30 (33.3)	18 (32.1)	12 (35.3)	0.528
	Semi-urban	2 (2.2)	2 (3.6)	0 (0)	
	Rural	58 (64.4)	36 (64.3)	22 (64.7)	
Ever smoked	Yes	76 (84.4)	50 (89.3)	26 (76.5)	0.136
	No	14 (15.6)	6 (10.7)	8 (23.5)	
Systolic BP (mmHg)		126±13	128±13	121±12	0.009
Diastolic BP (mmHg)		78±8	79±9	78±7	0.468
BP Status	Normotensive	60 (66.7)	34 (60.7)	26 (76.5)	0.239
	Pre-HTN	2 (2.2)	1 (1.8)	1 (2.9)	
	Hypertensive	28 (31.1)	21 (37.5)	7 (20.6)	
BMI (kg/m^2)		26.7±4.0	27.0±4.4	26.2±3.2	0.374
Duration of DM (years)		8.4±5.4	8.2±5.7	8.7±5.0	0.622
Diabetic drugs	OAD	49 (54.4)	30 (53.6)	19 (55.9)	1.000
	OAD + Insulin	41 (45.6)	26 (46.4)	15 (44.1)	
Metformin daily dose (gm)		1.0 (1.0-1.5)	1.0 (1.0-1.65)	1.0 (1.0-1.5)	0.471
Metformin duration (years)		7.0 (4.0-12.0)	5.0 (2.25-10.0)	8.5 (7.0-14.0)	0.006
Metformin gram-years		8.75 (3.5-13.7)	5.75 (2.0-13.6)	12.0 (7.9-14.0)	0.005
TCNS		8 (6-11)	8 (6-10)	10 (6-12)	0.296
Neuropathy	No	8 (8.9)	5 (8.9)	3 (8.8)	0.631
	Mild	42 (46.7)	29 (51.8)	13 (38.2)	
	Moderate	18 (20.0)	10 (17.9)	8 (23.5)	
	Severe	22 (24.4)	12 (21.4)	10 (29.4)	
HbA1c		8.3±1.2	8.3±1.2	8.3±1.2	0.762
S. Creatinine (mg/dL)		1.15±0.18	1.15±0.18	1.15±0.18	0.927
Estimated GFR (mL/min/1.73m^2)		64.0 (54.3-75.8)	64.5 (54.3-75.0)	63.5 (53.3-76.1)	0.958
Hemoglobin (gm/dL)		12.2±1.5	12.4±1.5	12.0±1.6	0.313
Anemia	Present	43 (47.8)	28 (50)	15 (44.1)	0.666
	Absent	47 (52.2)	28 (50)	19 (55.9)	
MCV (fL)		83.7±6.9	82.4±6.4	85.9±7.2	0.020
Total Cholesterol (mg/dL)		173.2±14.3	174.8±14.3	170.6±14.1	0.181
Triglyceride (mg/dL)		221.4±31.2	222.9±33.9	218.9±26.8	0.554
LDL Cholesterol (mg/dL)		142.9±16.6	143.2±16.9	142.4±16.4	0.823
HDL Cholesterol (mg/dL)		44.5±4.9	43.8±5.3	45.7±4.1	0.082

P-values by Student's *t*-test, Chi-square test, or Mann-Whitney U test as applicable.

Vitamin B12 is a water-soluble vitamin; foods, mainly those of animal protein origin, are the principal sources of this vitamin.[17] Vitamin B12 is essential in synthesizing the neuronal myelin sheath and synthesizing monoamines or neurotransmitters like serotonin and dopamine.[18] The hallmarks of vitamin B12 deficiency-induced neuronal damage includes axonal demyelination, degeneration, and later death; all these clinically manifest as severe peripheral or autonomic neuropathy, subacute combined degeneration of the spinal cord, delirium, and dementia.[19] Besides, vitamin B12 deficiency produces hyperhomocysteinemia, which is an independent risk factor for atherosclerotic disease.[13]

Metformin is a biguanide that is recommended by the American Diabetes Association (ADA), European Association for the Study of Diabetes (EASD), and International Diabetes Federation (IDF) as initial medical therapy for type 2 diabetes at diagnosis.[2,3,20] Metformin is perennially reported as a pharmacological cause of vitamin B12 deficiency.[4-10,21] The underlying mechanism is mostly controversial; proposed contributors have included competitive inhibition or inactivation of B12 absorption, alterations in intrinsic factor levels, gut bacterial flora, gastrointestinal motility, or ileal morphological structure, and interaction with the cubulin endocytic receptor.[21,22] Metformin is also shown to impair calcium-dependent membrane activity in the ileum, including uptake of the B12-intrinsic factor complex.[23]

In the study, 37.8% had subnormal (6.7% deficiency and 31.1% borderline) serum vitamin B12 levels. The reported prevalence of vitamin B12 deficiency after metformin use is 5% to 40%, varying with race, metformin usage, and cut-off values for defining B12

Table 2: Correlation of serum vitamin B12 level with other variables

Variables	*r*	*P*
Age (years)	0.029	0.783
Duration of DM (years)	-0.103	0.332
Metformin daily dose (mg)	-0.001	0.994
Metformin duration (years)	-0.377	<0.001
Metformin gram-years	-0.338	0.001
Systolic BP (mmHg)	0.275	0.009
Diastolic BP (mmHg)	0.143	0.179
BMI (kg/m^2)	0.098	0.360
TCNS	-0.062	0.559
HbA1c	-0.048	0.653
S. Creatinine (mg/dL)	0.007	0.951
eGFR (mL/min/1.73 m^2)	-0.034	0.752
Hemoglobin (gm/dL)	0.011	0.920
MCV (fL)	-0.272	0.010
Total Cholesterol (mg/dL)	0.057	0.592
Triglyceride (mg/dL)	-0.035	0.746
LDL Cholesterol (mg/dL)	0.034	0.750
HDL Cholesterol (mg/dL)	-0.067	0.533

By Pearson correlation test

status.[10] We had a lower frequency of B12 deficiency than the reported prevalence. A previous small-scale study in Bangladesh reported a 14% (seven out of 50) frequency of B12 deficiency in otherwise healthy non-diabetic subjects.[24] Moreover, large-scale studies conducted in this subcontinent reported a high (12% to 67%) prevalence of B12 deficiency in the general population.[25] So, it is very tough to comment on whether B12 deficiency is more prevalent in our patients with T2DM than in the general population. Only more extensive can studies incorporating a comparison group may answer this debate.

Previous observational studies and meta-analyses have demonstrated duration- and dose-dependent decrease in serum vitamin B12 concentrations in T2D patients using metformin.[6,8,26-29] Though even short-term treatment with metformin causes a decrease in serum B12 leading to peripheral neuropathy in T2DM, the exact duration of metformin use causing B12 deficiency is a matter of debate.[26] Like the previous researchers, we observed strong negative correlations of serum B12 levels with the duration of metformin use and gram-years of metformin use. The study subjects with subnormal B12 also used metformin for a longer duration, and gram-years of metformin use were higher than those with normal B12 levels. In contrast to the previous findings, we did not find any relationship between metformin dose with B12 status; this may be because of the small sample size.

The clinical significance of B12 deficiency lies in peripheral neuropathy and megaloblastic anemia. As metformin use is associated with B12 deficiency, metformin use may increase the risk of anemia in T2DM.[30] In this study, Hb levels were similar in normal and subnormal B12 groups. Moreover, Hb did not correlate with serum B12 level. Hb level in a patient with DM may be affected by factors other than diabetes, including age, nutrition, and other causes of anemia.[30] In this study, MCV was found higher in the subjects with subnormal B12, and the B12 level showed a strong negative correlation with MCV. Reinstatler *et al.* had similar observations.[5]

Though neuropathy due to metformin-induced B12 deficiency has been studied and reported numerous times, their causal relationships remain unclear.[8] We observed no differences in TCNS and frequency of neuropathy between the normal B12 and subnormal B12 groups. Diagnosis of diabetic neuropathy was associated with lower levels of B12 in previous studies.[10,11] It is hardly possible to distinguish neuropathy caused by B12 deficiency from diabetic neuropathy clinically. Therefore, neuropathy should still be closely monitored, and other causes of neuropathy should be searched in a patient with DM having neuropathy since early diagnosis and treatment improve prognosis.[30]

Limitations of the study

The small sample size was small; the findings may not be generalizable to the general population. It is a cross-sectional study; therefore, an actual cause--effect relationship between metformin use and B12 deficiency cannot be established. We did not measure serum homocysteine and serum methylmalonic acid levels, the functional markers of B12 deficiency, which may better reflect the B12 status than serum B12 levels. The diagnosis of peripheral neuropathy was clinical; electrophysiological study and nerve biopsy were not done.

Conclusion

A substantial portion of the metformin users in this study had subnormal vitamin B12. Higher duration of metformin use and gram-years of metformin use were associated with B12 deficiency. Patients with T2DM should be tested for serum vitamin B12 test periodically to diagnose and treat B12 deficiency at the earliest time.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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