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Original Research Article

PREVALENCE OF VITAMIN B12 DEFICIENCY AND ITS ASSOCIATION WITH THE DURATION OF METFORMIN THERAPY IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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ABSTRACT

Background: Vitamin B12 deficiency is an important nutritional complication associated with prolonged metformin therapy in patients with type 2 diabetes mellitus (T2DM). It may contribute to neurological and haematological abnormalities, particularly among patients receiving long-term or high-dose metformin treatment. The aim is to determine the prevalence of vitamin B12 deficiency and evaluate its association with the duration and dosage of metformin therapy among patients with type 2 diabetes mellitus.

Materials and Methods: This hospital-based observational cross-sectional study included 114 patients with T2DM receiving metformin therapy at a tertiary care hospital. Demographic characteristics, diabetes duration, metformin dosage, treatment duration, clinical manifestations, and biochemical parameters were recorded. Serum vitamin B12 concentrations were measured using a chemiluminescent immunoassay. Levels below 200 pg/mL were classified as deficient. Statistical analysis was performed using SPSS version 28.0, with $p<0.05$ considered statistically significant.

Results: Vitamin B12 deficiency was observed in 32 patients (28.07%), while 25 (21.93%) had borderline concentrations. Deficiency increased significantly with metformin treatment duration, from 11.90% among patients treated for less than five years to 51.72% among those treated for more than ten years ($p=0.001$). Similarly, deficiency increased from 10.71% in patients receiving less than 1000 mg/day to 42.50% among those receiving more than 1500 mg/day ($p=0.015$). Peripheral neuropathy (68.75% versus 24.39%), anaemia (56.25% versus 20.73%), and macrocytosis (37.50% versus 8.54%) were significantly more frequent among vitamin B12-deficient patients (all $p<0.001$).

Conclusion: Vitamin B12 deficiency was associated with longer metformin therapy duration, higher daily dosage, and increased neurological and haematological abnormalities. Periodic vitamin B12 monitoring may facilitate early detection and appropriate management among patients receiving prolonged metformin treatment.

Keywords: Vitamin B12 deficiency; Type 2 diabetes mellitus; Metformin therapy; Treatment duration; Peripheral neuropathy.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from insulin resistance, progressive pancreatic β-cell dysfunction, and impaired glucose metabolism. It represents a major public health concern because of its increasing prevalence, long-term complications, and considerable healthcare burden. Effective management requires sustained glycaemic control through lifestyle modifications and pharmacological

interventions. Metformin, an oral biguanide, remains one of the most widely prescribed medications for managing T2DM because of its efficacy, affordability, favourable safety profile, and low risk of hypoglycaemia. However, prolonged metformin therapy has been associated with nutritional disturbances, particularly vitamin B12 deficiency, which may contribute to neurological and haematological complications.[1] Vitamin B12, also known as cobalamin, is a water-soluble vitamin essential for DNA synthesis, erythropoiesis, neurological function, and cellular metabolism. It serves as an important cofactor in methionine synthesis and methylmalonyl-CoA metabolism, thereby contributing to normal red blood cell production and maintenance of the myelin sheath. Vitamin B12 deficiency may develop gradually and remain clinically unrecognized because hepatic stores can delay the appearance of symptoms. Clinical manifestations include fatigue, weakness, megaloblastic anaemia, paraesthesia, sensory disturbances, impaired vibration sensation, and peripheral neuropathy. In patients with diabetes, neurological manifestations of vitamin B12 deficiency may resemble diabetic peripheral neuropathy, creating diagnostic difficulties and potentially delaying appropriate management.[2] The relationship between metformin therapy and vitamin B12 deficiency is clinically important because metformin is frequently continued for several years after the diagnosis of T2DM. Although its exact mechanism remains incompletely understood, metformin-associated vitamin B12 deficiency is believed to involve impaired intestinal absorption of the vitamin B12–intrinsic factor complex, possibly through interference with calcium-dependent absorption in the terminal ileum. Alterations in intestinal motility and gastrointestinal physiology may also contribute. The development of deficiency is influenced by several interacting factors, including age, dietary habits, duration of diabetes, nutritional status, and concurrent medication use. Consequently, assessment of vitamin B12 status requires consideration of both treatment-related and patient-specific characteristics.[3] The duration of metformin therapy is considered an important factor influencing vitamin B12 status because prolonged exposure may progressively interfere with intestinal absorption and contribute to depletion of body stores. Patients receiving metformin for extended periods may remain asymptomatic despite declining serum vitamin B12 concentrations. In addition to treatment duration, daily metformin dosage and cumulative drug exposure may influence susceptibility to deficiency. The relationship between metformin therapy and peripheral neuropathy is particularly important because neurological complications may compromise mobility, functional independence, and quality of life. Therefore, evaluating treatment duration and dosage alongside serum vitamin B12 concentrations may help identify patients who require closer biochemical monitoring and early clinical assessment.[4] Early detection of vitamin B12 deficiency among metformin-treated patients is an important consideration in long-term diabetes management. Serum vitamin B12 measurement is commonly used for initial assessment, although borderline concentrations may require additional evaluation using functional biomarkers such as methylmalonic acid or homocysteine. Routine clinical assessment should also consider symptoms suggestive of anaemia or neuropathy, especially among individuals receiving prolonged therapy. The possibility of progressive vitamin B12 depletion emphasizes the importance of reviewing medication history and cumulative metformin exposure during follow-up visits. Incorporating nutritional evaluation and appropriate laboratory investigations into diabetes care may facilitate timely recognition of deficiency and reduce the likelihood of potentially preventable complications.[5] Several clinical characteristics may modify the relationship between metformin use and vitamin B12 deficiency. Increasing age, longer diabetes duration, restricted consumption of animal-derived foods, gastrointestinal disorders, and concomitant medications affecting vitamin absorption may contribute to reduced vitamin B12 availability. Haematological parameters, including haemoglobin concentration and mean corpuscular volume, provide additional information regarding possible deficiency-related abnormalities. Glycated haemoglobin reflects long-term glycaemic control and may help characterize the metabolic profile of affected patients. Nevertheless, the presence of normal haematological indices does not exclude vitamin B12 deficiency, and neurological manifestations may occur without overt macrocytic anaemia. A comprehensive clinical and biochemical evaluation is therefore necessary when investigating vitamin B12 status in patients receiving metformin.[6]

MATERIALS AND METHODS

The present study was designed as a hospital-based observational cross-sectional study to assess the prevalence of vitamin B12 deficiency and its association with the duration of metformin therapy among patients with type 2 diabetes mellitus (T2DM). The study also evaluated the relationship between serum vitamin B12 levels and demographic characteristics, glycaemic control, metformin dosage, haematological parameters, and clinical manifestations of vitamin B12 deficiency. The study was conducted at a tertiary care hospital in the Department of General Medicine in collaboration with the Department of Biochemistry. Patients diagnosed with type 2 diabetes mellitus and receiving metformin therapy were recruited from the outpatient and inpatient departments. The study was conducted after obtaining approval from the

Institutional Ethics Committee, and written informed consent was obtained from all participants. A total of 114 patients diagnosed with type 2 diabetes mellitus who were receiving metformin therapy were included in the study. Patients fulfilling the eligibility criteria were enrolled using a consecutive sampling technique. The participants were evaluated for serum vitamin B12 levels, duration and dosage of metformin therapy, glycaemic status, and associated clinical and biochemical parameters.

Inclusion Criteria

Patients aged 30 years or above with an established diagnosis of type 2 diabetes mellitus who had been receiving metformin therapy for at least six months were included. Patients receiving metformin alone or in combination with other oral antidiabetic medications or insulin were considered eligible. Only patients willing to participate and provide written informed consent were enrolled.

Exclusion Criteria

Patients with type 1 diabetes mellitus, previous vitamin B12 deficiency requiring treatment, recent vitamin B12 supplementation, pernicious anaemia, known malabsorption syndromes, previous gastric or ileal surgery, severe chronic kidney disease, chronic liver disease, or other conditions significantly affecting vitamin B12 absorption or metabolism were excluded. Pregnant women and patients receiving medications known to substantially interfere with vitamin B12 metabolism, other than metformin, were also excluded.

Data Collection and Clinical Assessment: Detailed demographic and clinical information was collected using a structured proforma. Parameters included age, gender, body mass index (BMI), dietary habits, duration of diabetes, family history of diabetes, associated comorbidities, and history of smoking and alcohol consumption. Information regarding metformin therapy included daily dosage, duration of treatment, frequency of administration, treatment adherence, and concomitant antidiabetic medications. History of proton pump inhibitor use and other medications potentially influencing vitamin B12 levels was also recorded. Clinical examination included measurement of height, weight, blood pressure, and assessment of peripheral neuropathy. Symptoms such as fatigue, generalized weakness, paraesthesia, numbness, impaired vibration sensation, and gait disturbances were documented.

Assessment of Metformin Therapy: The duration of metformin therapy was calculated from the date of treatment initiation to the date of patient evaluation using medical records and prescription history. Patients were categorized into three groups according to treatment duration: less than 5 years, 5–10 years, and more than 10 years. Daily metformin dosage was recorded in milligrams and further categorized as less than 1000 mg/day, 1000–1500 mg/day, and more than 1500 mg/day. The association of treatment duration and daily dosage with vitamin B12 deficiency was evaluated.

Biochemical and Haematological Investigations: Venous blood samples were collected under standard aseptic precautions for laboratory investigations. Serum vitamin B12 concentration was measured using a chemiluminescent immunoassay. Additional investigations included fasting blood glucose, postprandial blood glucose, glycated haemoglobin (HbA1c), complete blood count, haemoglobin concentration, mean corpuscular volume (MCV), serum creatinine, and estimated glomerular filtration rate (eGFR). Haematological abnormalities, including anaemia and macrocytosis, were evaluated to determine their association with serum vitamin B12 concentrations.

Classification of Vitamin B12 Status: Vitamin B12 status was classified according to serum concentrations. Levels below 200 pg/mL were considered deficient, levels between 200 and 300 pg/mL were classified as borderline, and levels above 300 pg/mL were considered normal. The prevalence of vitamin B12 deficiency was calculated as the proportion of patients with serum levels below 200 pg/mL among the total study population. The frequency of vitamin B12 deficiency was compared across different categories of metformin treatment duration and dosage.

Outcome Measures: The primary outcome was the prevalence of vitamin B12 deficiency among patients with type 2 diabetes mellitus receiving metformin therapy and its association with treatment duration. Secondary outcomes included the relationship between vitamin B12 levels and daily metformin dosage, age, gender, duration of diabetes, BMI, HbA1c, haemoglobin concentration, MCV, dietary habits, and clinical manifestations suggestive of peripheral neuropathy.

Statistical Analysis: Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics version 28.0. Categorical variables were expressed as frequencies and percentages, whereas continuous variables were presented as mean ± standard deviation or median with interquartile range, depending on data distribution. Normality was assessed using the Shapiro–Wilk test. The Chi-square test or Fisher's exact test was applied for categorical comparisons. The independent Student's t-test, Mann–Whitney U test, one-way ANOVA, or Kruskal–Wallis test was used for continuous variables, as appropriate. Spearman's correlation coefficient was used to assess the relationship between metformin duration and serum vitamin B12 levels. Multivariable logistic regression was planned to identify independent factors associated with vitamin B12 deficiency, subject to an adequate number of outcome events. Adjusted odds ratios with 95% confidence intervals were calculated. A p-value less than 0.05 was considered statistically significant.

RESULTS

[Table 1] Comparison of Baseline Demographic and Clinical Characteristics According to Vitamin B12 Deficiency Status (N=114)

[Table 1] presents the demographic and clinical characteristics of 114 patients with type 2 diabetes mellitus, of whom 32 had vitamin B12 deficiency and 82 were non-deficient. The largest proportion of patients belonged to the 50–59 years age group (35.09%), followed by those aged ≥60 years (32.46%). Vitamin B12 deficiency was more frequently observed among patients aged ≥60 years (43.75% of the deficient group), although the association with age was statistically insignificant (p=0.343). Males constituted 57.89% of the study population, with no significant gender-based difference in vitamin B12 deficiency (p=0.534). Among deficient patients, 37.50% had a BMI ≥30 kg/m² compared with 20.73% of non-deficient patients, although this difference was statistically insignificant (p=0.116). A significantly greater proportion of vitamin B12-deficient patients had diabetes for more than 10 years (56.25% versus 30.49%; p=0.020). Vegetarian dietary habits were significantly associated with vitamin B12 deficiency (62.50% versus 39.02%; p=0.024), whereas hypertension showed no statistically significant association (p=0.257).

[Table 2] Distribution and Prevalence of Vitamin B12 Deficiency Among Patients Receiving Metformin Therapy (N=114)

[Table 2] illustrates the distribution of serum vitamin B12 concentrations among 114 patients with type 2 diabetes mellitus receiving metformin therapy. Vitamin B12 deficiency, defined as a serum concentration below 200 pg/mL, was identified in 32 patients, representing an overall prevalence of 28.07%. Borderline vitamin B12 concentrations between 200 and 300 pg/mL were observed in 25 patients (21.93%), while 57 patients (50.00%) had normal vitamin B12 levels exceeding 300 pg/mL. The mean serum vitamin B12 concentration among deficient patients was 153.20 ± 25.80 pg/mL, compared with 251.40 ± 26.10 pg/mL among patients with borderline concentrations. Patients with normal vitamin B12 status demonstrated a considerably higher mean concentration of 432.60 ± 97.20 pg/mL. The overall mean serum vitamin B12 concentration in the study population was 314.44 ± 142.46 pg/mL.

[Table 3] Association Between Duration of Metformin Therapy and Vitamin B12 Deficiency (N=114)

[Table 3] demonstrates the association between the duration of metformin therapy and vitamin B12 deficiency among the study participants. Of the 114 patients, 42 (36.84%) had received metformin for less than 5 years, 43 (37.72%) for 5–10 years, and 29 (25.44%) for more than 10 years. Vitamin B12 deficiency was observed in 5 patients (11.90%) receiving metformin for less than 5 years, whereas 12 patients (27.91%) receiving treatment for 5–10 years were deficient. The highest prevalence of vitamin B12 deficiency was observed among patients receiving metformin for more than 10 years, with 15 patients (51.72%) demonstrating deficiency. Conversely, the proportion of patients without vitamin B12 deficiency decreased from 88.10% in the less-than-5-year group to 48.28% in the more-than-10-year group. The association between metformin treatment duration and vitamin B12 deficiency was statistically significant (p=0.001).

Table 1: Comparison of Baseline Demographic and Clinical Characteristics According to Vitamin B12 Deficiency Status (N=114)

Parameters	Total (N=114)	B12 Deficient (n=32)	Non-Deficient (n=82)	p-value
Age groups (years)				0.343
30–39	12 (10.53%)	2 (6.25%)	10 (12.20%)	
40–49	25 (21.93%)	5 (15.62%)	20 (24.39%)	
50–59	40 (35.09%)	11 (34.38%)	29 (35.37%)	
≥60	37 (32.46%)	14 (43.75%)	23 (28.05%)	
Gender				0.534
Male	66 (57.89%)	20 (62.50%)	46 (56.10%)	
Female	48 (42.11%)	12 (37.50%)	36 (43.90%)	
BMI (kg/m²)				0.116
<25	38 (33.33%)	7 (21.88%)	31 (37.80%)	
25–29.9	47 (41.23%)	13 (40.62%)	34 (41.46%)	
≥30	29 (25.44%)	12 (37.50%)	17 (20.73%)	
Duration of diabetes (years)				0.020
<5	26 (22.81%)	3 (9.38%)	23 (28.05%)	
5–10	45 (39.47%)	11 (34.38%)	34 (41.46%)	
>10	43 (37.72%)	18 (56.25%)	25 (30.49%)	
Dietary habits				0.024
Vegetarian	52 (45.61%)	20 (62.50%)	32 (39.02%)	
Mixed diet	62 (54.39%)	12 (37.50%)	50 (60.98%)	
Hypertension				0.257
Present	58 (50.88%)	19 (59.38%)	39 (47.56%)	
Absent	56 (49.12%)	13 (40.62%)	43 (52.44%)	

Table 2: Distribution and Prevalence of Vitamin B12 Deficiency Among Patients Receiving Metformin Therapy (N=114)

Vitamin B12 Status	Serum Vitamin B12 Level (pg/mL)	Number of Patients	Percentage (%)	Mean Serum Vitamin B12 (pg/mL)
Deficient	<200	32	28.07%	153.20 ± 25.80
Borderline	200–300	25	21.93%	251.40 ± 26.10
Normal	>300	57	50.00%	432.60 ± 97.20
Total	—	114	100.00%	314.44 ± 142.46

Table 3: Association Between Duration of Metformin Therapy and Vitamin B12 Deficiency (N=114)

Duration of Metformin Therapy	Total Patients n (%)	Vitamin B12 Deficient n (%)	Non-Deficient n (%)	p-value
<5 years	42 (36.84%)	5 (11.90%)	37 (88.10%)	
5–10 years	43 (37.72%)	12 (27.91%)	31 (72.09%)	
>10 years	29 (25.44%)	15 (51.72%)	14 (48.28%)	
Total	114 (100.00%)	32 (28.07%)	82 (71.93%)	0.001

Table 4: Association Between Daily Metformin Dosage and Vitamin B12 Deficiency (N=114)

Daily Metformin Dosage	Total Patients n (%)	Vitamin B12 Deficient n (%)	Non-Deficient n (%)	p-value
<1000 mg/day	28 (24.56%)	3 (10.71%)	25 (89.29%)	
1000–1500 mg/day	46 (40.35%)	12 (26.09%)	34 (73.91%)	
>1500 mg/day	40 (35.09%)	17 (42.50%)	23 (57.50%)	
Total	114 (100.00%)	32 (28.07%)	82 (71.93%)	0.015

Table 5: Comparison of Clinical Manifestations, Haematological and Biochemical Parameters According to Vitamin B12 Status (N=114)

Parameters	Vitamin B12 Deficient (n=32)	Non-Deficient (n=82)	p-value
Clinical manifestations			
Peripheral neuropathy	22 (68.75%)	20 (24.39%)	<0.001
Fatigue/generalized weakness	19 (59.38%)	27 (32.93%)	0.010
Anaemia	18 (56.25%)	17 (20.73%)	<0.001
Macrocytosis	12 (37.50%)	7 (8.54%)	<0.001
Impaired vibration sensation	16 (50.00%)	10 (12.20%)	<0.001
Haematological parameters			
Haemoglobin (g/dL), mean ± SD	11.42 ± 1.28	12.76 ± 1.39	<0.001
Mean corpuscular volume (fL), mean ± SD	95.60 ± 9.10	86.30 ± 7.20	<0.001
Biochemical parameters			
HbA1c (%), mean ± SD	8.42 ± 1.22	7.89 ± 1.27	0.044
eGFR (mL/min/1.73 m²), mean ± SD	75.10 ± 16.30	78.20 ± 17.40	0.374

[Table 4] Association Between Daily Metformin Dosage and Vitamin B12 Deficiency (N=114)

[Table 4] presents the relationship between daily metformin dosage and the prevalence of vitamin B12 deficiency among patients with type 2 diabetes mellitus. Among 114 participants, 28 (24.56%) were receiving metformin at a daily dosage below 1000 mg, 46 (40.35%) were receiving 1000–1500 mg/day, and 40 (35.09%) were receiving more than 1500 mg/day. Vitamin B12 deficiency was observed in only 3 patients (10.71%) receiving less than 1000 mg/day, compared with 12 patients (26.09%) receiving 1000–1500 mg/day. The highest prevalence of deficiency was recorded among patients receiving more than 1500 mg/day, affecting 17 patients (42.50%). The proportion of patients without vitamin B12 deficiency declined progressively from 89.29% in the lowest dosage group to 57.50% in the highest dosage group. A statistically significant association was observed between increasing daily metformin dosage and vitamin B12 deficiency (p=0.015).

[Table 5] Comparison of Clinical Manifestations, Haematological and Biochemical Parameters According to Vitamin B12 Status (N=114)

[Table 5] compares clinical manifestations, haematological findings, and biochemical parameters between patients with and without vitamin B12 deficiency. Peripheral neuropathy was significantly more frequent among vitamin B12-deficient patients than non-deficient patients (68.75% versus 24.39%; p<0.001), while fatigue or generalized weakness was observed in 59.38% and 32.93%, respectively (p=0.010). Anaemia was significantly more prevalent among deficient patients (56.25% versus 20.73%; p<0.001), along with macrocytosis (37.50% versus 8.54%; p<0.001) and impaired vibration sensation (50.00% versus 12.20%; p<0.001). The mean haemoglobin concentration was significantly lower in deficient patients (11.42 ± 1.28 g/dL) compared with non-deficient patients (12.76 ± 1.39 g/dL; p<0.001). Conversely, mean corpuscular volume was significantly higher in deficient patients (95.60 ± 9.10 fL versus 86.30 ± 7.20 fL; p<0.001). Mean HbA1c was also significantly higher in the deficient group (8.42 ± 1.22% versus 7.89 ± 1.27%; p=0.044), whereas the difference in eGFR was statistically insignificant (p=0.374).

DISCUSSION

The present study evaluated 114 patients with type 2 diabetes mellitus receiving metformin therapy, including 32 patients (28.07%) with vitamin B12 deficiency. The majority of patients belonged to the 50–59 years age group (35.09%), followed by those aged ≥60 years (32.46%). Although 43.75% of vitamin B12-deficient patients were aged ≥60 years, age was not significantly associated with deficiency (p=0.343). Similarly, gender (p=0.534) and BMI (p=0.116) showed no significant associations. However, diabetes duration exceeding 10 years was significantly more frequent among deficient patients than non-deficient patients (56.25% versus 30.49%; p=0.020). These findings are comparable to those reported by Huynh et al. (2024), who studied 156 metformin-treated patients and found vitamin B12 deficiency in 18.60%. Their study demonstrated a significant association between diabetes duration and vitamin B12 deficiency (OR=1.12; 95% CI: 1.03–1.19), whereas age (p=0.91), gender (p=0.73), and BMI (p=0.44) were not significantly associated. The similarity suggests that diabetes duration and associated cumulative treatment exposure may be more relevant than individual demographic characteristics in determining vitamin B12 status.[7]

Dietary habits were significantly associated with vitamin B12 deficiency in the present study, with vegetarian patients constituting 62.50% of the deficient group compared with 39.02% of the non-deficient group (p=0.024). This observation highlights the potential contribution of reduced dietary vitamin B12 intake among patients receiving prolonged metformin therapy. Comparable findings were reported by Ting et al. (2006), who conducted a nested case-control study involving 155 patients with metformin-related vitamin B12 deficiency and 310 matched controls. Vegetarian dietary habits were observed in 2.60% of deficient patients compared with 0.30% of controls (p=0.04). Their adjusted analysis demonstrated that vegetarianism was associated with increased odds of vitamin B12 deficiency (OR=16.20; 95% CI: 1.69–154.00), although the wide confidence interval indicated considerable uncertainty. The investigators also found no significant gender difference between the groups (p=0.42), consistent with the present findings. These observations suggest that dietary patterns should be considered alongside metformin exposure when evaluating vitamin B12 deficiency.[8]

The overall prevalence of vitamin B12 deficiency in the present study was 28.07%, with 32 patients having serum concentrations below 200 pg/mL. Borderline vitamin B12 concentrations of 200–300 pg/mL were identified in 25 patients (21.93%), while 57 patients (50.00%) demonstrated normal concentrations. The mean serum vitamin B12 concentration was 153.20 ± 25.80 pg/mL among deficient patients, compared with 251.40 ± 26.10 pg/mL in the borderline group and 432.60 ± 97.20 pg/mL among patients with normal vitamin B12 status. These findings are comparable to those of Shivaprasad et al. (2020), who evaluated 2,887 patients with type 2 diabetes mellitus and reported vitamin B12 concentrations below 200 pg/mL in 24.50% of metformin users compared with 17.30% of non-metformin users. Borderline concentrations between 200 and 300 pg/mL were recorded in 34.50% and 22.60%, respectively (p<0.001). The present study demonstrated a slightly higher prevalence of definite deficiency but a lower prevalence of borderline deficiency among metformin users, potentially reflecting differences in nutritional characteristics and cumulative treatment exposure.[9]

The combined prevalence of deficient and borderline vitamin B12 concentrations in the present study was 50.00%, indicating that half of the metformin-treated patients had serum vitamin B12 concentrations of 300 pg/mL or below. The overall mean vitamin B12 concentration was 314.44 ± 142.46 pg/mL. Similar observations were reported by Pflipsen et al. (2009), who conducted a cross-sectional study involving 203 patients with type 2 diabetes mellitus and identified metabolically confirmed vitamin B12 deficiency in 22.00% (44 patients). They observed significantly lower serum vitamin B12 concentrations among metformin users compared with non-users (425.99 versus 527.49 pg/mL; p=0.012). Their investigation also demonstrated a significant association between metformin use and biochemical evidence of vitamin B12 deficiency (p=0.04). Although the prevalence in the present study was higher, both studies demonstrate an appreciable burden of vitamin B12 deficiency among patients with diabetes. Differences in prevalence may be attributed to variations in diagnostic criteria, as Pflipsen et al. incorporated methylmalonic acid and homocysteine measurements to confirm deficiency.[10]

A significant association between metformin therapy duration and vitamin B12 deficiency was observed in the present study (p=0.001). The prevalence of deficiency increased progressively from 11.90% among patients receiving metformin for less than 5 years to 27.91% among those receiving treatment for 5–10 years and 51.72% among patients treated for more than 10 years. These findings are supported by Ko et al. (2014), who investigated 799 patients with type 2 diabetes mellitus receiving metformin therapy and identified vitamin B12 deficiency in 9.50% (76 patients). Compared with patients receiving metformin for less than 4 years, the adjusted odds of deficiency were significantly greater among those receiving treatment for 4–10 years (OR=4.65; 95% CI: 2.36–9.16) and those treated for at least 10 years (OR=9.21; 95% CI: 3.38–25.11), with p<0.001 for both comparisons. Despite differences in overall prevalence and diagnostic thresholds, the progressively increasing frequency of deficiency

with prolonged treatment exposure was consistent with the present study.[11]

The association between longer metformin exposure and vitamin B12 deficiency was further demonstrated by the present study, in which 15 of 29 patients (51.72%) treated for more than 10 years had vitamin B12 deficiency, compared with only 5 of 42 patients (11.90%) receiving treatment for less than 5 years. These findings are consistent with the longitudinal investigation conducted by Aroda et al. (2016) in the Diabetes Prevention Program Outcomes Study. At five years, low vitamin B12 concentrations were significantly more frequent in the metformin group than the placebo group (4.30% versus 2.30%; p=0.02). Combined low and borderline-low vitamin B12 concentrations were also more frequent among metformin users at five years (19.10% versus 9.50%; p<0.01) and at 13 years (20.30% versus 15.60%; p=0.02). Furthermore, every additional year of metformin exposure was associated with a 13% increase in the odds of vitamin B12 deficiency (OR=1.13; 95% CI: 1.06–1.20). Although the study populations differed, these findings support the importance of cumulative metformin exposure in vitamin B12 depletion.[12]

The present study also demonstrated a statistically significant association between daily metformin dosage and vitamin B12 deficiency (p=0.015). The prevalence of deficiency was 10.71% among patients receiving less than 1000 mg/day, increased to 26.09% among those receiving 1000–1500 mg/day, and reached 42.50% among patients receiving more than 1500 mg/day. Similar dose-related findings were reported by Kim et al. (2019), who conducted a cross-sectional study involving 1,111 metformin-treated patients with type 2 diabetes mellitus. Vitamin B12 deficiency was identified in 22.20% of patients (247 cases). Compared with patients receiving less than 1000 mg/day, the adjusted odds ratios for deficiency were 1.72 for those receiving 1000–1500 mg/day, 3.34 for those receiving 1500–2000 mg/day, and 8.67 among patients receiving at least 2000 mg/day. The associations were significant for the two highest dosage categories (p<0.001). These findings correspond closely with the dose-related pattern observed in the present study and indicate that higher daily metformin exposure may contribute substantially to vitamin B12 deficiency.[13]

Neurological manifestations were significantly more frequent among vitamin B12-deficient patients in the present study. Peripheral neuropathy was observed in 68.75% of deficient patients compared with 24.39% of non-deficient patients (p<0.001). Similarly, impaired vibration sensation was identified in 50.00% and 12.20% of patients, respectively (p<0.001), while fatigue or generalized weakness was present in 59.38% and 32.93% (p=0.010). However, contrasting findings were reported by Ahmed et al. (2016), who studied 121 metformin-treated patients with type 2 diabetes mellitus and reported an overall vitamin B12 deficiency prevalence of 28.10%, which was almost identical to the 28.07% observed in the present study. Despite this similarity, neuropathy was observed in 32.30% of vitamin B12-deficient patients and 36.80% of patients with normal vitamin B12 concentrations, with no significant association between vitamin B12 status and neuropathy. The discrepancy may reflect differences in neuropathy assessment, diabetes severity, and other contributing neurological risk factors. These findings emphasize that peripheral neuropathy among patients with diabetes may have multiple causes and cannot be attributed solely to vitamin B12 deficiency.[14]

Haematological abnormalities were significantly associated with vitamin B12 deficiency in the present study. Anaemia was observed in 56.25% of deficient patients compared with 20.73% of non-deficient patients (p<0.001), whereas macrocytosis was present in 37.50% and 8.54%, respectively (p<0.001). The mean haemoglobin concentration was significantly lower among deficient patients (11.42 ± 1.28 versus 12.76 ± 1.39 g/dL; p<0.001), while mean corpuscular volume was significantly higher (95.60 ± 9.10 versus 86.30 ± 7.20 fL; p<0.001). These findings can be compared with those of Al-Fawaeir et al. (2022), who investigated 155 patients with type 2 diabetes mellitus, including 120 metformin users and 35 non-users. They reported significantly lower mean vitamin B12 concentrations among metformin users (268.50 ± 35.80 versus 389.50 ± 29.80 pg/mL; p=0.029). Mean corpuscular volume was also higher in metformin users (87.50 ± 2.90 versus 83.70 ± 2.40 fL), although this difference was not statistically significant. In the present study, mean HbA1c was additionally higher among deficient patients (8.42 ± 1.22% versus 7.89 ± 1.27%; p=0.044), whereas eGFR showed no significant difference (p=0.374). Overall, the findings suggest that vitamin B12 deficiency may coexist with important haematological and metabolic abnormalities among metformin-treated patients.[15]

CONCLUSION

The present study demonstrated a considerable prevalence of vitamin B12 deficiency (28.07%) among patients with type 2 diabetes mellitus receiving metformin therapy. Vitamin B12 deficiency showed a significant association with longer duration of metformin therapy and higher daily metformin dosage. Deficient patients also exhibited a higher frequency of peripheral neuropathy, anaemia, macrocytosis, and impaired vibration sensation. These findings highlight the importance of periodic vitamin B12 monitoring, particularly among patients receiving long-term or high-dose metformin therapy. Early identification and appropriate management of vitamin B12 deficiency may help prevent associated neurological and haematological complications.

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