Research Paper

Association of Vitamin B12 Deficiency and Clinical Neuropathy with Metformin use in Individuals with type 2 Diabetes

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ABSTRACT:

BACKGROUND:

Type II diabetes mellitus (T2DM) is linked to chronic microvascular complications, including neuropathy, nephropathy, and retinopathy. Diabetic peripheral neuropathy (DPN) affects almost half of all patients and can lead to serious problems like foot ulcers and amputations. A lack of vitamin B12 can make neurological problems worse by causing axonal demyelination, which could lead to permanent nerve damage.

PATIENTS AND METHOD:

This cross-sectional observational study was performed at the Iraqi National Centre for Diabetes & Endocrinology to evaluate serum vitamin B12 levels in T2DM patients treated with metformin compared to other oral hypoglycemic agents, and to investigate the correlation with peripheral neuropathy.

RESULTS:

A total of 89 patients (44 males and 45 females, mean age 56 years) were enrolled, with 63 receiving metformin and 26 receiving alternative therapies. Blood samples taken after fasting were used to measure vitamin B12, glucose, and HbA1c levels. The average serum B12 level was much lower in people who took metformin ($P < 0.01$), but there were no big differences in BMI, FBS, or HbA1c. People who took metformin had diabetes for an average of 2.6 years longer ($P < 0.01$). The majority of metformin users (92.06%) were consuming 1000–2000 mg daily. In total, 7.8% of the group had a vitamin B12 deficiency. Metformin users were more likely to have a deficiency than non-users (9.5% vs. 3.8%, $P < 0.036$). While metformin correlated with an increased occurrence of mild to moderate peripheral neuropathy, no substantial association was identified between B12 deficiency and neuropathy.

CONCLUSION:

The study concludes that metformin use is significantly associated with vitamin B12 deficiency in type 2 diabetes patients

KEYWORDS: Vitamin B12, deficiency, clinical Neuropathy, metformin, type 2 Diabetes.

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INTRODUCTION:

Diabetes mellitus (DM) is a heterogeneous group of metabolic disorders characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. It affects the metabolism of carbohydrates, fats, and proteins, with classic symptoms including polyuria, polydipsia, unexplained weight loss, fatigue, and blurred vision. However, these manifestations may be absent or subtle, and hyperglycemia may already be causing pathological changes before clinical diagnosis is established [1]. The two principal forms of diabetes are type I diabetes mellitus (T1DM), an autoimmune disorder involving β-cell destruction and insulin deficiency, and type II diabetes mellitus (T2DM), a more prevalent form that results from insulin resistance and relative insulin deficiency [2]. T2DM constitutes the majority of global diabetes cases and is strongly associated with obesity, sedentary lifestyle, and genetic predisposition [3]. The pathophysiology of T2DM involves impaired insulin action in liver, muscle, and adipose tissue, as well as reduced insulin secretion from pancreatic β-cells, culminating in sustained hyperglycemia and increased risk of complications [4]. Hyperglycemia contributes to long-term damage and failure of various organs, particularly the eyes, kidneys, nerves, and cardiovascular system. These complications are categorized into microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (cardiovascular disease, stroke, and peripheral vascular disease) complications [5]. Pharmacological interventions for glycemic control in T2DM have evolved significantly, with metformin—an oral biguanide—being widely recommended as the first-line agent. Metformin exerts its antihyperglycemic effect primarily by suppressing hepatic gluconeogenesis and improving insulin sensitivity in peripheral tissues, through mechanisms involving MP-activated protein kinase (AMPK) activation and mitochondrial respiratory chain inhibition [6]. Even though metformin works well and is safe, it has been linked to vitamin B12 deficiency, which is a possible but often ignored side effect [7]. Vitamin B12 (cobalamin) is a necessary water-soluble vitamin that plays a role in DNA synthesis, methylation, and the function of the nervous system. It is a coenzyme for methionine synthase and methylmalonyl-CoA mutase, which are both very important for keeping the nervous and blood systems working normally [8]. People mostly get vitamin B12 from foods that come from animals, and the process of absorbing it is complicated and needs gastric intrinsic factor and ileal receptor-mediated uptake [9]. A lack of vitamin B12 can cause megaloblastic anaemia, neuropsychiatric disorders, and permanent peripheral neuropathy because of axonal demyelination [10]. It is estimated that 10–30% of patients undergoing long-term metformin therapy may experience vitamin B12 deficiency [11]. The proposed mechanisms include impaired absorption due to metformin-induced interference with calcium-dependent uptake of the vitamin B12–intrinsic factor complex in the ileum [12], and possibly altered hepatic redistribution [13]. Given the overlapping symptomatology between diabetic peripheral neuropathy and B12 deficiency-related neuropathy, the clinical importance of recognizing and managing this complication is profound. Early detection of vitamin B12 deficiency in metformin-treated patients is critical to prevent irreversible neurological damage.

Aim of the Study: 1) to evaluate serum vitamin B12 levels in patients with type II diabetes mellitus receiving either metformin or other oral antidiabetic therapies, 2) to assess the impact of metformin dosage and duration of therapy on serum vitamin B12 concentrations, 3) to examine the correlation between vitamin B12 levels and the occurrence of peripheral neuropathy in individuals with type II diabetes.

PATIENTS AND METHODS:

This cross-sectional observational study was conducted at the *Iraqi National Center for Diabetes and Endocrinology* over a three-month period from September to November 2020. A total of 89 patients diagnosed with type II diabetes mellitus (T2DM) were enrolled, comprising 44 males and 45 females, with a mean age of 56 years (range: 42–80 years). Among them, 63 patients were receiving metformin therapy, while 26 were treated with other oral hypoglycemic agents. All participants provided written informed consent prior to inclusion.

Study Design and Sample Selection:
The study did not report a formal sample size calculation or statistical power analysis prior to data collection. Instead, all eligible patients meeting the inclusion criteria during the study period were recruited, suggesting a consecutive sampling approach. Inclusion criteria were: a confirmed diagnosis of T2DM, current treatment with either metformin or non-metformin oral agents, and the presence of peripheral neuropathy signs as determined by a clinical neurologist. Patients receiving vitamin B12 supplements or multivitamins were excluded from the study. No adjustments were made for potential confounding variables such as dietary vitamin B12 intake, gastrointestinal disorders (e.g., malabsorption syndromes), use of proton pump inhibitors or H2 blockers, alcohol consumption, or other medications affecting vitamin B12 absorption.

The Toronto Clinical Neuropathy Score (TCSS) was used to check for peripheral neuropathy. It looks at symptoms like pain, numbness, and tingling, as well as reflexes and sensory modalities. According to TCSS, participants were put into four groups: no neuropathy (score 0–5), mild (6–9), moderate (10–12), and severe (>12). Furthermore, a targeted neurological examination was conducted. The study fails to indicate whether neuropathy evaluations were conducted by multiple assessors or to provide inter-rater reliability metrics, thereby constraining the evaluation of diagnostic consistency.

Clinical and Laboratory Measurements:
Anthropometric measurements included weight and height, with body mass index (BMI) calculated in kg/m² according to WHO classifications. Fasting venous blood samples (5 mL) were obtained after at least 10 hours of fasting. Three milliliters of blood were collected in gel tubes for glucose and vitamin B12 analysis, and 2 mL in EDTA tubes for HbA1c

measurement. Glucose and HbA1c levels were measured using the Roche Cobas® C111 analyzer, with HbA1c expressed as a percentage based on DCCT/NGSP standards. Serum vitamin B12 concentrations were determined using the Roche Cobas® e411 electrochemiluminescence immunoassay system, which is based on competitive binding with intrinsic factor. The cut-off for vitamin B12 deficiency was 140 pmol/L (189 pg/mL).

Statistical analysis: SPSS version 24 was used to analyse the data. Continuous variables were represented as mean ± standard deviation (SD), and group comparisons were performed utilising the independent t-test. The Chi-square test or Fisher's exact test were used to look at categorical variables, depending on how many cells there were. The results tables sometimes talk about both tests, but they don't say which test was used for each variable. A p-value of less than 0.05 was seen as statistically significant.

RESULTS:

This study enrolled 89 individuals with T2DM, dividing them into two groups according to their Oral Antidiabetic treatment: the first group comprised those taking metformin (metformin Subjects) and the second group consisted of those not taking metformin (non-metformin Subjects), as illustrated in Table 1.

The group of subjects taking metformin had an average age of 56.4 ±0.7 years, while the group of subjects not taking metformin had an average age of 55.8 ± 1.3 years. There were no significant differences in age or sex between the two groups (P = 0.3 and 0.1, respectively). The two groups did not differ significantly in weight and BMI (P = 0.2 and 0.7, respectively). But the average level of vitamin B12 in the blood was lower in the metformin group (P < 0.01). The average levels of FBS and HbA1c were not significantly different between the two groups (P = 0.6 and 0.2, respectively). The average duration of diabetes was approximately 2.6 years longer among metformin users (P < 0.01). Most of the people who took metformin (38; 60.3%) had been taking it for more than 4 years, while 24 (38.09%) had been taking it for up to 4 years, and only 1 person (1.58%) had been taking it for less than 1 year. The majority of metformin users (58; 92.06%) were administered a daily dosage of 1000–2000 mg, while 3 (4.7%) and 2 (3.1%) received an average daily dosage exceeding 2000 mg and falling below 1000 mg, respectively.

Table 1: Demographics characteristics of Subjects.

Characteristics	Group		*P*-value
	Non-metformin user (n = 26) Mean (SD) or n (%)	Metformin user (n = 63) Mean (SD) or n (%)	
Gender			
Male	14 (52.69)	30 (46.10)	*0.3*
Female	12 (74.31)	33 (53.90)	
Age (years)	55.8 ± 1.3	56.4 ± 0.7	*0.1*
Vitamin B12 Level (pmol/L)	358.32±186.78	315.67±139.64	*0.01*
FBS	10.25±4.61	9.88±5.24	*0.589*
HbA1c	8.22±1.71	8.15±1.45	*0.197*
Weight	84.1 ± 1.9	82.0 ± 1.3	*0.2*
BMI§	32.5 ± 0.7	31.8 ± 0.7	*0.70*
Duration of Diabetes (years)	6.8 ± 0.7	9.9 ± 0.5	*<0.01*
Duration of Metformin Intake (years)			
< 1	0	1 (1.58)	*n/a*
1–4	0	24 (38.09)	
> 4	0	38 (60. 3)	
Metformin Dose (mg)			
< 1,000	0	2 (3.1)	*n/a*
1,000–2,000	0	58 (92.06)	
> 2,000	0	3 (4.76)	
Toronto Clinical Scoring System			
No Neuropathy	21 (80.76)	44 (69.84)	*0.12*
Mild	4 (15.38)	14(22.22)	
Moderate	1 (3.84)	5 (7.93)	
Focused Neurological Test			
Negative	22 (84.62)	55 (87.30)	*0.72*
Positive	4 (15.38)	8 (12.69)	

The overall prevalence of vitamin B12 deficiency among all individuals with type 2 diabetes mellitus was 7.8% (7 out of 89), as shown in Table 2. Metformin subjects exhibited a markedly higher prevalence of vitamin B12 deficiency compared to non-metformin subjects (9.5% vs. 3.8%, P < 0.036).

Table 2: A comparison of the percentage of patients with vitamin B12 deficiency between metformin users and non-users.

Variables	All participants (N = 89) n (%)	Non-metformin user (n = 26) n (%)	Metformin user (n = 63) n (%)	P-value
Normal serum vitamin B12	82 (92.23%)	25 (97.8%)	57 (90.6%)	< 0.036
Deficient serum vitamin B12	7 (7.77%)	1 (3.8%)	6 (9.50%)	

In comparison to the non-metformin group, the metformin group demonstrated a higher frequency of mild and moderate neuropathy (mild status: 22.22% vs. 15.38%; moderate status: 7.93% vs. 3.84%, respectively). This is illustrated in Figure 1 (A, B)
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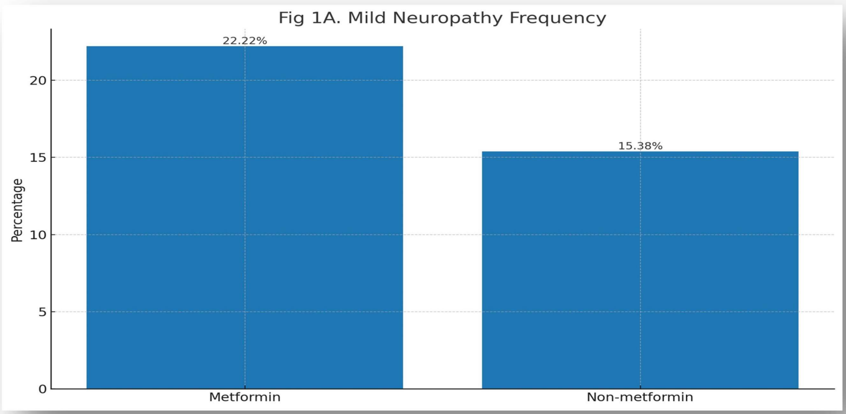

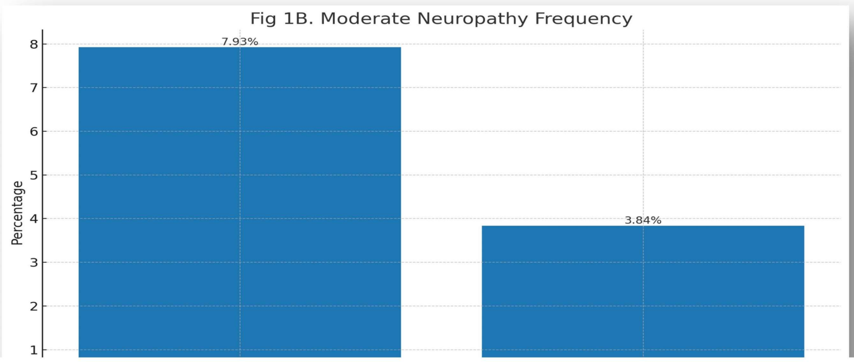

The clinical characteristics of the serum B12 deficient group and the serum B12-normal group are illustrated. The mean BMI disparity between the two groups was not statistically significant (P = 0.06), as shown in table 3. Furthermore, the TCSS and focused neuropathy test results were not statistically significant, with TCSS values of 0.96 (0.42, 2.34) and 0.18 (0.01, 3.16), and focused neurological test results of 1.45 (0.42, 4.99). The vitamin B12 deficiency group exhibited a substantially prolonged duration of treatment (P < 0.01) and a higher average daily dose of metformin (P < 0.01) than the groups without vitamin B12 deficiency. In terms of metformin dose, the odds ratios (ORs) were 3.69 (0.13, 92.70), 4.11 (0.94, 16.84), and 31.4 (5.29, 197.61), respectively. The ORs for metformin duration were 2.45 (0.46, 11.75) and 6.25 (1.57, 23.89).

Table 3: Clinical characteristics of the participants stratified by B12 deficiency status (N = 89).

Characteristic	Vitamin B12 deficiency		OR (95% CI) *	*P* value
	Yes (n =7) Mean (SD) or n (%)	No (n = 82) Mean (SD) or n (%)		
BMI	35.1 ± 1.25	32.2 ± 0.45		0.060
Metformin Dose (mg)				
0	1 (14.28)	25 (30.4)	Ref.	
< 1,000	0 (0)	2 (2.4)	3.69 (0.13, 92.70)	0.20
1,000–2,000	2 (28.57)	58 (70.73)	4.11 (0.94, 16.84)	0.05
> 2,000	4 (57.14)	3 (3.65)	31.4 (5.29, 197.61)	<0.01
Duration of Metformin Intake (years)				
0	1 (14.28)	25 (30.4)	Ref.	
< 4	2 (28.57)	23 (28.04)	2.45 (0.46, 11.75)	0.47
> 4	4 (57.14)	34 (41.46)	6.25 (1.57, 23.89)	<0.01
Toronto Clinical Scoring				
No Neuropathy	4 (57.14)	61(74.39)	Ref.	
Mild	3(42.85)	15(18.29)	0.96 (0.42, 2.34)	1.00
Moderate	0 (0)	6 (7.31)	0.18 (0.01, 3.16)	0.28
Focused Neurological test				0.78
Negative	6(85.71)	71 (86.85)	Ref.	
Positive	1(14.28)	11 (13.41)	1.45 (0.42, 4.99)	0.60

*** OR: Odds ratio, CI: Confidence Interval**

DISCUSSION:

Metformin remains the most widely prescribed oral antidiabetic drug for the management of type 2 diabetes mellitus (T2DM) due to its proven efficacy, safety profile, and cardiovascular benefits. However, increasing evidence has raised concerns about its association with vitamin B12 deficiency. The findings from this study support these concerns, showing a higher prevalence of vitamin B12 deficiency among metformin users (9.52%) compared to those not on metformin (3.8%), with an overall prevalence of 7.86%. These results align with previous studies demonstrating a significant relationship between metformin use and reduced serum vitamin B12 levels [14-16]. Importantly, vitamin B12 deficiency was more commonly observed in patients taking higher doses of metformin (>2000 mg/day) and those with a longer duration of use (>4 years). These findings are consistent with the results of Ko S-h et al. [17] and a systematic review by Chapman et al., which found that 17 cross-sectional studies reported an association between lower serum B12 levels and metformin therapy [18]. However, certain studies present divergent viewpoints, indicating that metformin predominantly influences the inactive variant of vitamin B12 (holo-haptocorrin) rather than the active variant (holotranscobalamin), which may constrain the clinical relevance of noted decreases in total B12 [19,20]. The present study identified a predominance of mild neuropathy among metformin users in the context of peripheral neuropathy, aligning with the findings

of Kamphuis et al. [21] while contrasting with studies
that indicated more severe neuropathic manifestations [22]. Gupta et al. (2018) found that the severity of neuropathy was positively related to how long a person had been taking metformin. This suggests that long-term treatment may make neuropathy worse [23]. Interestingly, studies have reported neuroprotective effects of metformin independent of glycemic control. For example, Ma J et al. demonstrated that metformin reduced chemotherapy-induced peripheral neuropathy (CIPN) [24], which shares clinical features with diabetic neuropathy [25]. Other experimental models have shown that metformin may reverse neuropathic pain [25], inhibit neuronal apoptosis [26,27], and stimulate neurogenesis [28,29]. These findings highlight the dual nature of metformin's influence on neuropathy: it may either protect neuronal tissue or contribute to neuropathic symptoms through vitamin B12 depletion. The absence of a significant association between vitamin B12 deficiency and peripheral neuropathy in this study does not rule out its pathogenic role. Rather, it suggests that metformin's impact on neuropathy may result from a complex interplay of mechanisms, potentially explaining the variability in findings across different studies. This study's cross-sectional design limits the ability to establish causal relationships between metformin use, vitamin B12 deficiency, and neuropathy [29]. The relatively small sample size may reduce statistical power and generalizability. Additionally, serum vitamin B12 levels were measured without assessing functional biomarkers such as methylmalonic acid or homocysteine, which could better reflect tissue-level deficiency.

CONCLUSION:

There is a significant relationship between metformin use and B12 deficiency. Metformin may lead to higher frequency of mild to moderate peripheral neuropathy. No significant relationship between b12 deficiency and peripheral neuropathy.

Conflict of Interest: The authors declare no conflict of interest.

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