ORIGINAL ARTICLE

To Determine Vitamin B12 Deficiency in Type 2 Diabetes Mellitus Patients on Metformin Therapy

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Abstract

Introduction: India harbors the second-largest population with diabetes, with over 100 million, and type 2 diabetes mellitus (T2DM) constitutes the major share. Metformin remains the first-line pharmacotherapy for T2DM due to its safety profile, cost-effectiveness, and beneficial metabolic effects.

Materials and methods: The aim of the study was to assess the frequency of vitamin B12 deficiency in patients with T2DM on metformin therapy and compare it with their cohabiting family members who are not on metformin but share similar dietary habits.

Results: This study included 180 participants with 90 cases and controls each, and we enrolled 89 females (49.4%) and 91 males (50.6%). The mean age was 57 (± 4.88) years, and overall gender distribution and dietary pattern were nearly balanced among cases and controls. The mean duration of diabetes among cases was 7.69 ± 4.35 years, and duration of metformin use was 5.22 ± 3.77 years, ranging from 1–16 years. The mean daily dose of metformin was 1238.89 ± 586.50 mg/day, with a median dose of 1000 mg/day. The mean serum vitamin B12 level in metformin users was significantly lower than in controls (206.66 ± 59.09 pg/mL vs 301.44 ± 72.28 pg/mL, $p < 0.001$). Vitamin B12 deficiency was present in 40.0% of metformin users versus 11.1% of controls, yielding an odds ratio of 5.33 (95% CI: 2.44–11.65), which was a highly significant difference between the two groups ($t = -9.631$, $p < 0.001$), strongly suggesting an association between metformin use and reduced B12 levels. Neurological symptoms were observed in 14.4% of cases (OR 4.896, 95% CI: 1.345–17.827; $p = 0.009$).

Conclusion: Long-term metformin use in T2DM patients is strongly associated with both biochemical vitamin B12 deficiency and an increased likelihood of neurological symptoms.

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Introduction

Diabetes mellitus (DM) is a long-term metabolic disorder with rapidly mounting global burden.[1] Globally, India ranks second in terms of the diabetic population, with over 100 million, and type 2 diabetes mellitus (T2DM) constitutes the major portion.[2] Metformin mainly acts by suppressing gluconeogenesis in the liver and improving insulin sensitivity.[3] According to prevailing guidelines, metformin is recommended as the initial agent for most T2DM patients, especially those who are overweight or obese with preserved renal function.[1–4] However, prolonged metformin therapy has been linked to vitamin B12 (cobalamin, VB12) deficiency. VB12 is a vital water-soluble nutrient involved in DNA synthesis, erythropoiesis, and neuroprotection. The absorption of vitamin B12 occurs in the terminal ileum after forming a complex with intrinsic factor (IF). Metformin may interfere with this absorption process by impairing the calcium-dependent mechanism that facilitates the uptake of the IF–B12 complex in the ileum and by altering intestinal motility.[5,6] Vitamin B12 deficiency can cause megaloblastic anemia and neurological manifestations, including neuropathy, cognitive decline, and subacute combined degeneration of the spinal cord. Symptoms such as fatigue, memory loss, depression, and glossitis may also occur.[7,8] In addition to worsening diabetic neuropathy, B12 deficiency can exacerbate cardiovascular risk through elevated homocysteine levels. Hence, awareness, timely identification, and correction are of paramount importance. Recent studies have quantified this risk of VB12 deficiency and have estimated the prevalence in metformin users ranging from 8 to 30%.[5–9] In South Asia, widespread vegetarian dietary patterns further contribute to reduced vitamin B12 intake, amplifying the risk of deficiency. However, routine assessment and management may be inadequate, particularly in a resource-constrained and predominantly vegetarian population. The true burden of this deficiency in real-world Indian diabetic cohorts and its differentiation from dietary or other environmental causes remains poorly characterized.

Hence, we planned this study to bridge this gap and to isolate the pharmacological impact of metformin from dietary and genetic confounders. We aimed to evaluate the frequency of Vitamin B12 deficiency among individuals with T2DM who are undergoing metformin treatment and to compare it with their cohabiting family members not on metformin but sharing similar dietary habits. The primary objective was to determine the prevalence of vitamin B12 deficiency in T2DM patients who have been receiving metformin either as monotherapy or alongside other antidiabetic medications for a duration exceeding one year. The secondary objective was to compare the vitamin B12 status of T2DM patients with their household family members who share similar dietary practices but are not exposed to metformin.

Materials and Methods

This was designed as a case-control, observational study following approval from the institutes' ethics committee for academic research projects vide approval number ECARP/2024/25, dated 25/09/2024 at a tertiary care teaching public hospital. The total study duration was 18 months, commencing from 01/10/2024. The objective of the study was to determine the prevalence of vitamin B12 deficiency in T2DM patients who have been receiving metformin either as monotherapy or alongside other antidiabetic medications for a duration exceeding 1 year and compare it with their cohabiting family members who are not on metformin but share similar dietary habits. All the patients of T2DM on metformin therapy attending the outpatient department (OPD) of a public hospital with their cohabiting family member during the study period were screened and enrolled in the study. Written informed

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consent was obtained from all participating patients and their respective family members after explaining the purpose, procedure, and confidentiality of the study. We included adult patients 18 years and above of either sex. For cases, patients of T2DM on metformin for more than 1 year as monotherapy or in combination with other drugs were enrolled. For controls, family members of the above patients, who were nondiabetic and not on metformin, residing in the same household and sharing similar dietary habits, were selected. We excluded type 1 DM, patients diagnosed with inflammatory bowel disease, those with a history of gastrectomy or colectomy, chronic liver or kidney disorders, malignancies, long-term alcohol use, or any other condition known to influence vitamin B12 levels. Patients on vitamin B12 supplementation were also excluded from the study.

After thorough screening according to the predetermined inclusion and exclusion criteria, participants received a thorough explanation of the study protocol, followed by the acquisition of written informed consent. Each participant's involvement in the study was limited to a single outpatient visit. This included the informed consent process, detailed history, examination of the patients, collection of demographic and clinical data, and blood sampling for hemogram and serum vitamin B12 analysis. For the cases group, blood sampling was done as a standard of care, and for the control group, it was done only for the study purpose. Data gathered from each participant encompassed their demographic characteristics, comprehensive clinical history, comorbidities, duration of T2DM, duration and dose of metformin, general and detailed multisystem examination, including neuropathy evaluation. Blood sampling was done following the standard aseptic procedure and sent to the laboratory for hemogram, including hemoglobin (Hb), mean corpuscular volume (MCV), and serum vitamin B12 levels. A threshold of <200 pg/mL, consistent with established diagnostic criteria, was used to define vitamin B12 deficiency.

The sample size was determined based on prevalence data reported by Kumar et al., applying the following formula.[10]

$n = Z^2 \times P \times (1-P)/E^2$ (where: $Z = 1.96$, $P = 0.2733$, $1-P = 0.7267$, $E = 0.10$ with acceptable margin of error) = 0.7627. Thus, sample size (n) = 76.27. Adjusting for a 10% dropout rate: $n\_adj = n/(1-d) = 76.27/(1-0.10) = 76.27/0.90 \approx 85$. Thus, the final sample size was rounded to 90 participants per group, making a total of 180 participants to ensure adequate power and accommodate potential dropouts.

Vitamin B12 was assessed at a single point in time among cases and controls rather than following individuals who were initially free of deficiency to see who later developed it; prevalence is the appropriate measure. Categorical variables were described using frequencies and percentages, whereas continuous variables were presented as mean ± standard deviation or median with interquartile range (IQR). Within the qualitative data, nominal variables comprised group classification (case/control), participant sex, metformin usage, and dietary habits (vegetarian/mixed), vitamin B12 deficiency (yes/no), and neurological symptoms. Associations between qualitative variables were evaluated using the Chi-square test, incorporating continuity correction for all 2 × 2 contingency tables. In cases where expected cell counts were too low to validate the Chi-square test, Fisher's exact test was employed for 2 × 2 tables. For tables with more than two rows and insufficient cell counts, adjacent rows were combined, and the Chi-square test was reapplied. Continuity correction was also applied to all 2 × 2 tables following data pooling—for instance, when examining the relationship between dietary patterns (vegetarian/ mixed) and group category (case/control). Quantitative variables were presented as mean ± standard deviation (SD) or as median with IQR. These included measures such as age, duration of diabetes mellitus in cases, metformin dose, duration of metformin use in cases, VB12 level, MCV, and hemoglobin level. Quantitative data were compared across the binary qualitative variable representing group classification (case/control) using the unpaired t-test if the data passed the "Shapiro–Wilk normality test," or the Mann–Whitney U test if the data failed the "normality" test. [e.g., comparison of vitamin B12 Level (pg/mL) between cases and controls]. Graphical representations were employed where appropriate to enhance the presentation of results. Statistical analyses were conducted using suitable software tools, including but not limited to MS Excel, Microsoft Office 365, and PSPP (version 2.0.1, released on 21 March 2024). A p-value threshold of ≤0.05 was considered indicative of statistical significance.

Results

This study included 180 participants with 90 cases and controls each. Overall, the study population was almost equally distributed with 89 females (49.4%) and 91 males (50.6%). The mean age of participants was 57 years (SD ± 4.88). The normality of age distribution was evaluated by applying the Shapiro–Wilk test ($p = 0.456$ for cases and $p = 0.142$ for controls; $p > 0.05$ for both) and an independent samples t-test assuming equal variances ($t = -1.158$, $p = 0.248$) revealed no statistically significant difference between the groups, confirming they were age-matched (Table 1). Dietary patterns were matched between the two groups, with 41 out of 90 participants (45.6%) in each group following a vegetarian diet and 49 out of 90 participants (54.4%) in each group having a mixed diet. The case and control groups were comparable in terms of age, sex, and dietary patterns, with no statistically significant differences observed between them.

Duration of DM (years), metformin dosage, and duration of metformin use (years) were assessed only in the case group (n = 90), since all individuals in the control group were nondiabetics by study design. The mean (SD) duration of DM among cases was 7.69 ± 4.35 years. The mean (SD) daily dose of metformin was 1238.89 ± 586.50 mg/day, with a median dose of 1000 mg/day. The average duration of metformin use was 5.22 years (SD ± 3.77), with a range spanning 1–16 years and an IQR of 6 years (Fig. 1). Assessment of normality using the Shapiro–Wilk test indicated that the data were not normally distributed for duration of diabetes ($p = 0.00805$, $p < 0.05$), metformin dose ($p < 0.00000001$), and duration of metformin ($p = 0.0000083$), respectively, indicating that the data is right-skewed. The mean MCV and Hb were 90.26 ± 4.24 fL, and 13.13 ± 1.19 gm/dL among the 90 cases and 89.35 ± 3.91 fL. and 13.40 ± 1.05 gm/dL in the 90 controls, respectively. The Shapiro–Wilk test demonstrated that MCV and Hb values were normally distributed in both groups. Various variables of both groups are compared in Table 1.

The mean serum vitamin B12 level was 206.66±59.09 pg/mL with an IQR of 90.47 pg/mL among the 90 cases, while controls had a value of 301.44 ± 72.28 pg/mL, with an IQR of 96.08 pg/mL (Fig. 2) The Shapiro–Wilk test for normality revealed that vitamin B12 levels were not normally distributed in the case group

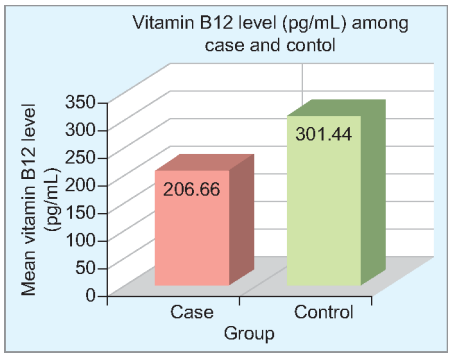

Fig. 1: Mean vitamin B12 values in cases and controls

Table 1: Various variables amongst cases and controls

	Groups	*Statistic*					
		Mean	*Std. deviation*	*Median*	*Interquartile range*	*Minimum*	*Maximum*
Age (years)	Case	56.956	4.690	57.000	6.000	44.000	67.000
	Control	57.778	4.834	58.000	6.250	48.000	71.000
Duration of diabetes mellitus (years)	Case	7.689	4.352	8.000	6.000	1.000	18.000
Metformin dose (mg/day)	Case	1238.889	586.495	1000.000	1500.000	500.000	2000.000
Duration of metformin use (years)	Case	5.222	3.768	4.500	6.000	1.000	16.000
Vitamin B12 level (pg/mL)	Case	206.658	59.086	213.387	90.471	110.550	330.656
	Control	301.439	72.283	308.900	96.075	134.000	459.300
MCV (fL)	Case	90.264	4.242	90.300	6.075	81.500	98.800
	Control	89.353	3.906	89.050	5.625	80.800	102.300
Hemoglobin (gm/dL)	Case	13.130	1.195	13.250	1.500	10.000	15.900
	Control	13.397	1.049	13.300	1.325	10.800	16.100

	Groups	*Shapiro–Wilk*		
		Statistic	*Significance*	*df*
Age (years)	Case	0.986	0.456	90
	Control	0.979	0.142	90
Duration of diabetes mellitus (years)	Case	0.961	0.00805	90
Metformin dose (mg/day)	Case	0.829	8.4911E-009	90
Duration of metformin use (years)	Case	0.907	8.3351E-006	90
Vitamin B12 level (pg/mL)	Case	0.961	0.00831	90
	Control	0.980	0.169	90
MCV (fL)	Case	0.986	0.451	90
	Control	0.978	0.134	90
Hemoglobin (gm/dL)	Case	0.992	0.837	90
	Control	0.989	0.646	90

t-test

	Groups	*N*	*Mean*	*Std deviation*	*Std error mean*
Age (years)	Case	90	56.96	4.69	0.49
	Control	90	57.78	4.83	0.51
Vitamin B12 level (pg/mL)	Case	90	206.66	59.09	6.23
	Control	90	301.44	72.28	7.62
MCV (fL)	Case	90	90.26	4.24	0.45
	Control	90	89.35	3.91	0.41
Hemoglobin (gm/dL)	Case	90	13.13	1.19	0.13
	Control	90	13.40	1.05	0.11

		t-test for equality of means	
		t	*Significance (2-tailed)*
Age (years)	Equal variances assumed	−1.158	0.248
Vitamin B12 level (pg/mL)	Equal variances assumed	−9.631	6.18E-018
MCV (fL)	Equal variances assumed	1.499	0.136
Hemoglobin (gm/dL)	Equal variances assumed	−1.591	0.113

($p = 0.00831$) but were normally distributed in the control group ($p = 0.169$). However, given the large sample size, the central limit theorem supported the use of a parametric test. An independent samples *t*-test, conducted under the assumption of equal variances, revealed a highly significant difference in serum vitamin B12 levels between the two groups ($t = -9.631$, $p < 0.001$), indicating a strong association between prolonged metformin therapy and reduced vitamin B12 concentrations (Table 1). Vitamin B12 deficiency was identified in 36 participants from the case group (40.0%) and in 10 individuals from the control group (11.1%) (Fig. 3). This indicates that the prevalence of deficiency among cases was over threefold higher than that in controls. Statistical evaluation using Pearson's Chi-square test revealed a highly significant difference between the groups ($\chi^2 = 19.740$, $df = 1$, $p < 0.001$), which was further substantiated by Fisher's exact test ($p = 1.31 \times 10^{-5}$). The calculated odds ratio exhibited that the odds of having vitamin B12 deficiency were 5.333 times higher in participants with T2DM on metformin therapy compared to their matched household controls (95% CI: 2.442–11.647) (Table 2). Neurological

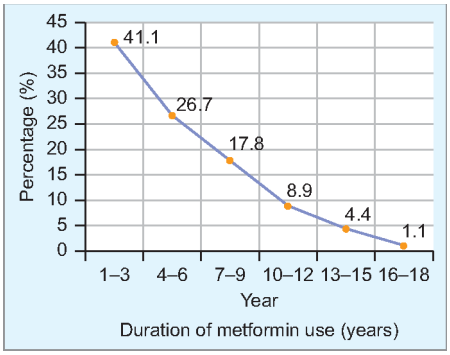

Fig. 2: Duration of metformin use (years) among cases

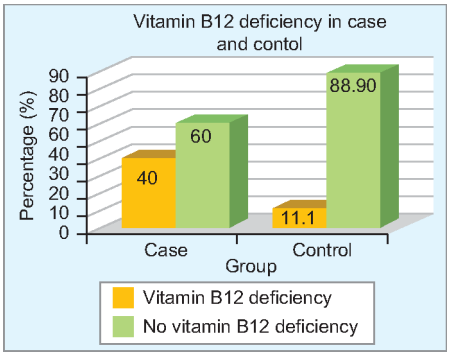

Fig. 3: Frequency of vitamin B12 deficiency among cases and controls

symptoms suggestive of B12 deficiency, including paresthesia, numbness, and balance disturbances, were noted in 14.4% of cases compared to 3.3% of controls (OR 4.896, 95% CI: 1.345–17.827; $p = 0.009$), underscoring the clinical impact of deficiency. The difference between the two groups was statistically significant (Pearson's Chi-square = 6.860, $df = 1$, $p = 0.009$; Fisher's exact test = 0.016) (Table 3). The odds of having neurological symptoms were 4.896 times higher in the cases compared to the controls (95% CI: 1.345–17.827), highlighting a significant correlation between metformin

Table 2: Vitamin B12 deficiency among study participants

			Group		Total
			Case	Control	
Vitamin B12 deficiency	Yes	Count	36	10	46
		% within group	40.0%	11.1%	25.6%
	No	Count	54	80	134
		% within group	60.0%	88.9%	74.4%
	Total	Count	90	90	180
		% within group	100.0%	100.0%	100.0%

	Value	df	Asymptotic significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)
Pearson Chi-square	19.740(b)	1	8.87E-006		
Continuity correction(a)	18.251	1	1.94E-005		
Likelihood ratio	20.677	1	5.44E-006		
Fisher's exact test				1.31E-005	6.55E-006
n of valid cases	180				

	Value	95% confidence interval	
		Lower	Upper
Odds ratio for group (case/control)	5.333	2.442	11.647
For cohort vitamin B12 deficiency = Yes	3.600	1.904	6.805
For cohort Vitamin B12 Deficiency = No	0.675	0.562	0.811
N of valid cases	180		

Table 3: Neurological symptoms among study participants

			Group		Total
			Case	Control	
Neurological symptoms	Yes	Count	13	3	16
		% within group	14.4%	3.3%	8.9%
	No	Count	77	87	164
		% within group	85.6%	96.7%	91.1%
Total		Count	90	90	180
		% within group	100.0%	100.0%	100.0%

	Value	df	Asymptotic significance (2-sided)	Exact significance (2-sided)	Exact significance (1-sided)
Pearson Chi-square	6.860(b)	1	0.00882		
Continuity correction(a)	5.556	1	0.01841		
Likelihood ratio	7.348	1	0.00671		
Fisher's exact test				0.016	0.008
N of valid cases	180				

	Value	95% confidence interval	
		Lower	Upper
Odds ratio for group (case/control)	4.896	1.345	17.827
For cohort neurological symptoms = Yes	4.333	1.278	14.691
For cohort neurological symptoms = No	0.885	0.806	0.971
N of valid cases	180		

therapy and the occurrence of neurological symptoms.

Discussion

This case-control study was conducted at a tertiary care hospital to determine the prevalence of vitamin B12 deficiency among patients with T2DM undergoing metformin therapy, in comparison to their nondiabetic household members who shared similar dietary patterns. The study enrolled a total of 180 participants, comprising 90 T2DM patients who had been on metformin for over 1 year and 90 matched controls without diabetes and not receiving metformin. The average age of participants was 57 years, with a SD of ±4.88, and overall gender distribution and dietary pattern were nearly balanced among cases and controls. The two groups were matched for age, sex, dietary habits, and living environment, thereby minimizing potential confounding factors. Demographic parameters were comparable with prior studies.[11,12] This middle-aged cohort likely reflects both the typical age of T2DM onset and the time required for chronic metformin exposure to result in clinically detectable vitamin B12 deficiency. However, dietary patterns from other studies show variability. Bora et al observed that the majority of their participants were nonvegetarians, while Agarwal et al. reported a predominance of vegetarian participants.[9,12] These discrepancies highlight the diversity influencing dietary practices across study populations. Animal-derived foods serve as the principal dietary source of vitamin B12, and it is essential to meticulously match dietary factors that strengthen the internal validity of the present study.

The mean (SD) duration of DM among cases was 7.69 ± 4.35 years, and duration of metformin use was 5.22 ± 3.77 years, ranging from 1–16 years. The mean (SD) daily dose of metformin was 1238.89 ± 586.50 mg/day, with a median dose of 1000 mg/day. Studies have reported consistent duration of DM and comparable mean daily dose.[13–16] The mean MCV and Hb were comparable among cases and controls. Majority studies mention similar findings; however, a few did observe a high proportion of cases with low MCV, indicating possible coexisting iron deficiency and higher anemia prevalence among metformin users.[9,17–19] Together, these findings from the present study and comparable literature underscore that while vitamin B12 deficiency frequently occurs in patients on metformin therapy, macrocytosis and anemia are not consistent or sensitive markers, particularly in populations with coexisting nutritional deficiencies or chronic disease, biochemical screening to detect early deficiency is necessary.

The mean serum vitamin B12 level was 206.66 ± 59.09 pg/mL among the 90 cases, while controls had a value of 301.44 ± 72.28 pg/mL. Vitamin B12 deficiency was observed in 36 cases (40.0%) and in 10 controls (11.1%). The likelihood of vitamin B12 deficiency was 5.333 times greater among cases compared to controls. The present study captures the cumulative metabolic impact of both diabetes and metformin therapy, which may partly explain the higher prevalence of B12 deficiency observed. Deficiency is affected by duration of exposure, dose, and individual metabolic factors. A recent study reiterates that higher doses and longer durations of metformin was linked with lower vitamin B12 concentrations, and vitamin B12 screening is highly recommended.[20] Overall, the consistency between our results and other long-term metformin user cohorts suggests that our mean duration of ~5 years is representative of real-world chronic T2DM management, and cumulative duration, rather than just dose, serves as a key contributing factor in the vitamin B12 deficiency. B12 deficiency may remain clinically silent in terms of hematologic manifestations. Findings from the current study demonstrate a clear, statistically robust association between prolonged metformin therapy and decreased serum vitamin B12 concentration, both in absolute values and in prevalence of deficiency. The large effect size (OR > 5) is noteworthy and exceeds that reported in several previous studies, likely due to the careful matching of dietary patterns and living environments between cases and controls, which removed major confounders such as meat intake variability. Comparable trends have been observed in previous studies with variation in mean B12 levels due to diet patterns, duration, and dose of metformin, etc.[20–25] Neurological symptoms suggestive of B12 deficiency, including paresthesia, numbness, and balance disturbances, were observed in 14.4% of cases, and the odds of having neurological symptoms were 4.896 times higher in the cases. Therefore, individuals with T2DM undergoing prolonged metformin treatment are at increased risk of developing vitamin B12 deficiency and are more prone to manifest neurological symptoms. Because fewer participants exhibited neurological symptoms than biochemical deficiency, this may imply that neuropathic manifestations progress over time rather than appearing immediately. Multiple studies have consistently demonstrated a link between metformin therapy, vitamin B12 deficiency, and the emergence of neuropathic symptoms, though reported prevalence rates vary.[26–28] Overall, the combined evidence supports that metformin-associated B12 deficiency increases the likelihood of neuropathy, reinforcing the need for early detection and preventive supplementation. Current management involves oral or intramuscular replacement with cyanocobalamin or methylcobalamin. Comparison with literature demonstrated consistent trends linking metformin with lower B12 levels and neuropathy, though the effect varied depending on population characteristics, dietary background, and study design.[28]

A key strength of this study is its robust case-control design, incorporating meticulous matching of cases and controls, which enhances the validity of the observed associations. By selecting cohabiting family members as controls, the study effectively minimized variability in dietary vitamin B12 intake, environmental exposures, and socioeconomic factors, thereby isolating the effect of extended metformin use on serum vitamin B12 levels. The relatively large sample size for a single-center study provided adequate statistical power. The study has certain limitations. Being a single-center, observational case-control design and cross-sectional data, causal inference cannot be firmly established, and residual confounding from unmeasured variables, such as differences in physical activity, undiagnosed comorbidities, cannot be excluded. Neurological symptoms were assessed using clinical history without electrophysiological confirmation, which might underestimate subclinical neuropathy prevalence.

From this case-control study of 180 participants, we conclude that patients with T2DM receiving long-term metformin therapy exhibited a substantially higher prevalence of vitamin B12 deficiency than their matched household controls who shared comparable dietary habits. The two groups were comparable in age, sex, and dietary pattern, and the odds of developing vitamin B12 deficiency were 5.333 times greater among cases than controls. The study revealed a highly significant association between extended metformin therapy and diminished serum vitamin B12 levels, both in terms of absolute values and deficiency prevalence. The diabetic cohort was characterized by a long history of diabetes and varying doses and durations of metformin, suggesting cumulative exposure, and B12 deficiency was present without any hematological alterations. Odds of having

neurological symptoms consistent with vitamin B12 deficiency were 4.896 times higher in the cases, highlighting its clinical relevance. Hence, we may recommend that routine monitoring of vitamin B12 levels should be integrated into the standard management protocol for patients with T2DM receiving long-term metformin therapy.

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