

Research Article

Association of Serum Vitamin B12 Levels with Duration of Metformin Therapy in Type 2 Diabetes Mellitus

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ABSTRACT

Background: Metformin is widely prescribed for the management of type 2 diabetes mellitus (T2DM); however, prolonged therapy has been associated with impaired vitamin B12 absorption. This study aimed to evaluate the association between serum vitamin B12 levels and duration of metformin therapy in patients with T2DM.

Materials and Methods: This hospital-based, observational, cross-sectional study included 130 patients with T2DM receiving metformin for at least six months. Participants were categorised according to treatment duration into Group I (less than 2 years), Group II (2-5 years) and Group III (more than 5 years). Serum vitamin B12 concentrations were measured and classified as deficient (below 200 pg/mL), borderline (200-300 pg/mL) or normal (above 300 pg/mL). Group-wise comparisons and correlation analyses were planned using appropriate statistical tests.

Results: In the dataset, the mean age was 54.6 ± 10.8 years, with 56.2% males. Vitamin B12 deficiency was observed in 23.8% of patients, while 30.8% had borderline concentrations. Mean serum vitamin B12 levels progressively decreased across Groups I, II and III (402 ± 112 , 326 ± 105 and 241 ± 96 pg/mL, respectively; $p < 0.001$). Deficiency prevalence increased from 7.9% to 19.6% and 41.3% across the respective groups ($p < 0.001$).

Conclusion: The findings suggest an association between prolonged metformin therapy and reduced serum vitamin B12 concentrations. Periodic assessment may facilitate early identification of deficiency among patients receiving long-term treatment.

Keywords: Type 2 Diabetes Mellitus, Metformin, Vitamin B12 Deficiency, Treatment Duration, Cobalamin.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterised by persistent hyperglycaemia resulting from insulin resistance and progressive pancreatic β -cell dysfunction. It represents a major global health concern due to its increasing prevalence and associated microvascular and macrovascular complications. Metformin remains one of the most widely prescribed oral antihyperglycaemic agents owing to its established efficacy, affordability, favourable safety profile and minimal risk of hypoglycaemia. Its principal mechanisms include suppression of hepatic gluconeogenesis and improvement in peripheral insulin sensitivity. Consequently, many patients with T2DM receive metformin therapy for prolonged periods, either alone or in combination with other antidiabetic medications [1-3].

Despite its therapeutic benefits, long-term metformin administration has been associated with impaired vitamin B12 absorption. Vitamin

B12 (cobalamin) is an essential water-soluble vitamin involved in DNA synthesis, erythropoiesis, cellular metabolism and maintenance of normal neurological function. Its deficiency may lead to megaloblastic anaemia, peripheral neuropathy, cognitive impairment and other neurological manifestations, which may occur even in the absence of haematological abnormalities [4,5]. Although the exact mechanism remains incompletely understood, metformin is believed to interfere with calcium-dependent absorption of the intrinsic factor-vitamin B12 complex in the terminal ileum. Additional proposed mechanisms include alterations in intestinal motility and gut microbiota. These effects may progressively reduce vitamin B12 availability, particularly among individuals receiving higher cumulative doses or prolonged treatment. Since hepatic vitamin B12 stores may sustain physiological requirements for several years, biochemical deficiency can develop gradually and remain clinically unrecognised [6,7].

The relationship between metformin treatment duration and serum vitamin B12 concentration is of particular clinical interest. Previous observational studies have demonstrated an association between prolonged metformin exposure and reduced vitamin B12 levels. However, the magnitude of this association varies depending on treatment dosage, dietary habits, age, baseline nutritional status, concomitant medications and individual susceptibility [7–10]. Furthermore, neurological manifestations of vitamin B12 deficiency may overlap with diabetic peripheral neuropathy, potentially delaying diagnosis and contributing to additional neurological morbidity [5].

Evidence from long-term clinical investigations further supports this association. A randomised placebo-controlled trial by de Jager et al. demonstrated significantly reduced vitamin B12 concentrations following prolonged metformin administration [11]. Similarly, the Diabetes Prevention Program Outcomes Study reported an increasing risk of biochemical vitamin B12 deficiency with additional years of metformin exposure [12]. These findings emphasise the importance of evaluating vitamin B12 status in patients receiving long-term treatment, particularly those presenting with anaemia or peripheral neuropathy.

Considering the widespread use of metformin in T2DM, identifying the relationship between treatment duration and vitamin B12 status has considerable clinical relevance. Early recognition of deficiency may facilitate appropriate biochemical monitoring, timely supplementation when indicated and prevention of potentially avoidable complications. This is particularly important in settings where nutritional deficiencies may coexist with chronic metabolic disorders and routine vitamin B12 assessment is not consistently practised.

Therefore, the present study was undertaken to evaluate the association between serum vitamin B12 levels and duration of metformin therapy in patients with type 2 diabetes mellitus, with particular emphasis on determining whether prolonged treatment is associated with lower circulating vitamin B12 concentrations.

Grouping of Study Participants

Based on the duration of metformin therapy, patients were categorised into three groups:

Group	Duration of metformin therapy
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MATERIALS AND METHODS

The present hospital-based, observational, cross-sectional study was designed to evaluate the association between serum vitamin B12 levels and duration of metformin therapy among patients with type 2 diabetes mellitus (T2DM). The study was conducted in the Department of General Biochemistry at a tertiary care hospital. A total of 130 patients with an established diagnosis of T2DM receiving metformin therapy for at least six months were enrolled.

Inclusion Criteria

1. Patients aged 18 years and above with diagnosed T2DM.
2. Patients receiving metformin, either alone or in combination with other antidiabetic medications, for at least six months.
3. Patients willing to provide written informed consent.

Exclusion Criteria

1. Patients with type 1 diabetes mellitus or gestational diabetes.
2. Patients with previously diagnosed vitamin B12 deficiency or receiving vitamin B12 supplementation within the preceding six months.
3. Patients with pernicious anaemia, malabsorption syndromes or a history of gastrointestinal surgery affecting vitamin B12 absorption.
4. Patients with severe chronic liver disease or advanced renal dysfunction.
5. Patients receiving long-term proton pump inhibitors or histamine-2 receptor antagonists.
6. Pregnant and lactating women.

Study Procedure and Data Collection

Demographic and clinical information, including age, sex, dietary habits, body mass index (BMI), duration of diabetes, comorbidities, metformin dosage and duration of therapy, was recorded using a predesigned structured proforma. Metformin exposure was assessed through available prescription records and patient history. Clinical examination included assessment for symptoms suggestive of vitamin B12 deficiency, particularly fatigue, paraesthesia, numbness and peripheral neuropathy.

Group I	6 months to less than 2 years
Group II	2–5 years
Group III	More than 5 years

Laboratory Investigations

Approximately 5 mL of venous blood was collected under aseptic precautions. Serum vitamin B12 concentration was measured using

a chemiluminescent immunoassay (CLIA) method in the institutional biochemistry laboratory.

Vitamin B12 status was classified as follows:

Vitamin B12 status	Serum concentration
Deficient	Below 200 pg/mL
Borderline	200–300 pg/mL
Normal	Above 300 pg/mL

Additional investigations included fasting blood glucose, postprandial blood glucose, HbA1c, haemoglobin and mean corpuscular volume (MCV).

Outcome Measures

The primary outcome was the association between duration of metformin therapy and serum vitamin B12 concentration. Secondary outcomes included the prevalence of vitamin B12 deficiency, comparison of vitamin B12 levels across treatment-duration groups, and assessment of its relationship with metformin dosage, duration of diabetes and glycaemic control.

Statistical Analysis

Data were entered into Microsoft Excel and analysed using SPSS.21 Continuous variables were expressed as mean $\pm$ standard deviation (SD) or median (IQR), while categorical variables were presented as frequencies and percentages. Normality was assessed using the Shapiro–Wilk test. Intergroup comparisons were performed using one-way ANOVA or the Kruskal–Wallis test, as appropriate. Chi-square or Fisher's exact test was applied to categorical variables. Pearson's or Spearman's correlation analysis was used to assess the relationship between metformin duration and serum vitamin B12 levels. Multivariable regression was planned to adjust for relevant confounding factors. A p-value of less than 0.05 was considered statistically significant.

Results

A total of 130 patients with type 2 diabetes mellitus receiving metformin therapy were included in the present study. The baseline demographic and clinical characteristics, duration of metformin therapy, serum vitamin B12 status, and associations between vitamin

B12 levels and various clinical parameters were evaluated.

The mean age of the study participants was 54.6 ± 10.8 years, with the highest proportion belonging to the 51–60-year age group (34.6%), followed by those above 60 years (27.7%). Males constituted 56.2% (n=73) of the study population, while females accounted for 43.8% (n=57). The mean BMI was 26.4 ± 3.8 kg/m², and the mean duration of diabetes mellitus was 7.4 ± 4.2 years. The average daily metformin dosage was 1450 ± 486 mg. The mean HbA1c, haemoglobin, and mean corpuscular volume were $8.1 \pm 1.3\%$, 12.6 ± 1.5 g/dL, and 88.4 ± 7.6 fL, respectively (Table 1).

Based on the duration of metformin therapy, participants were divided into three groups. Group I (6 months to less than 2 years) comprised 38 patients (29.2%), whereas Group II (2–5 years) and Group III (more than 5 years) included 46 patients (35.4%) each. Thus, the majority of participants (70.8%) had been receiving metformin therapy for at least two years (Table 2 and Figure 1).

Serum vitamin B12 deficiency (below 200 pg/mL) was observed in 31 patients (23.8%), while 40 patients (30.8%) had borderline vitamin B12 levels (200–300 pg/mL). Normal vitamin B12 concentrations (above 300 pg/mL) were recorded in 59 patients (45.4%). Overall, 54.6% of the study population exhibited either deficient or borderline serum vitamin B12 levels (Table 3).

A progressive reduction in mean serum vitamin B12 concentration was observed with increasing duration of metformin therapy. Patients receiving metformin for less than two years (Group I) had the highest mean vitamin B12 concentration (402 ± 112 pg/mL), followed by Group II (326 ± 105 pg/mL). The lowest mean concentration was observed among

patients receiving metformin for more than five years (241 ± 96 pg/mL). The difference in mean serum vitamin B12 levels across the three groups was statistically significant ($p < 0.001$; one-way ANOVA), demonstrating an inverse association between duration of metformin therapy and serum vitamin B12 concentration (Table 4 and Figure 2).

The proportion of patients with vitamin B12 deficiency increased progressively with longer metformin exposure, from 7.9% in Group I to 19.6% in Group II and 41.3% in Group III. Similarly, borderline vitamin B12 status was observed in 21.1%, 32.6%, and 37.0% of patients in Groups I, II, and III, respectively. Conversely, the proportion of patients with normal vitamin B12 levels decreased from 71.1% in Group I to 47.8% in Group II and

21.7% in Group III. The association between duration of metformin therapy and serum vitamin B12 status was statistically significant ($p < 0.001$; Chi-square test) (Table 5).

The supplied Pearson's correlation coefficients demonstrated a significant negative correlation between serum vitamin B12 levels and duration of metformin therapy ($r = -0.52$, $p < 0.001$). Negative correlations were also reported with duration of diabetes mellitus ($r = -0.38$, $p < 0.001$), daily metformin dosage ($r = -0.31$, $p < 0.001$), age ($r = -0.19$, $p = 0.030$), and HbA1c ($r = -0.22$, $p = 0.012$). Although BMI also exhibited a weak negative correlation with serum vitamin B12 levels ($r = -0.11$), this association was not statistically significant ($p = 0.213$) (Table 6 and Figure 3).

RESULTS

Table 1. Baseline Demographic and Clinical Characteristics of Study Participants (N=130)

Parameter	Value
Age (years), mean $\pm$ SD	54.6 $\pm$ 10.8
Age distribution	
18–40 years	15 (11.5%)
41–50 years	34 (26.2%)
51–60 years	45 (34.6%)
Above 60 years	36 (27.7%)
Gender	
Male	73 (56.2%)
Female	57 (43.8%)
BMI (kg/m ²)	26.4 $\pm$ 3.8
Duration of diabetes (years)	7.4 $\pm$ 4.2
Daily metformin dosage (mg)	1450 $\pm$ 486
HbA1c (%)	8.1 $\pm$ 1.3
Haemoglobin (g/dL)	12.6 $\pm$ 1.5
Mean corpuscular volume (fL)	88.4 $\pm$ 7.6

Table 2. Distribution of Participants According to Duration of Metformin Therapy (N=130)

Duration of metformin therapy	Frequency (n)	Percentage (%)
Group I: 6 months to less than 2 years	38	29.2
Group II: 2–5 years	46	35.4
Group III: More than 5 years	46	35.4
Total	130	100.0

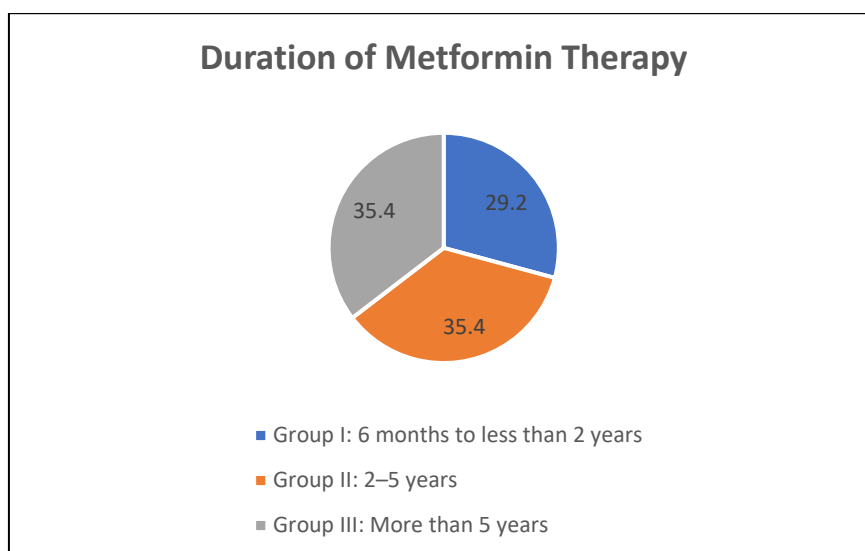


Figure 1. Distribution of Participants According to Duration of Metformin Therapy (N=130)

Table 3. Distribution of Study Participants According to Serum Vitamin B12 Status (N=130)

Vitamin B12 status	Serum concentration (pg/mL)	Frequency (n)	Percentage (%)
Deficient	Below 200	31	23.8
Borderline	200–300	40	30.8
Normal	Above 300	59	45.4
Total		130	100.0

Table 4. Comparison of Mean Serum Vitamin B12 Levels According to Duration of Metformin Therapy

Duration of metformin therapy	N	Serum vitamin B12 (pg/mL), Mean $\pm$ SD	p-value	Statistical test
Group I: Less than 2 years	38	402 $\pm$ 112	<0.001	One-way ANOVA
Group II: 2–5 years	46	326 $\pm$ 105		
Group III: More than 5 years	46	241 $\pm$ 96		

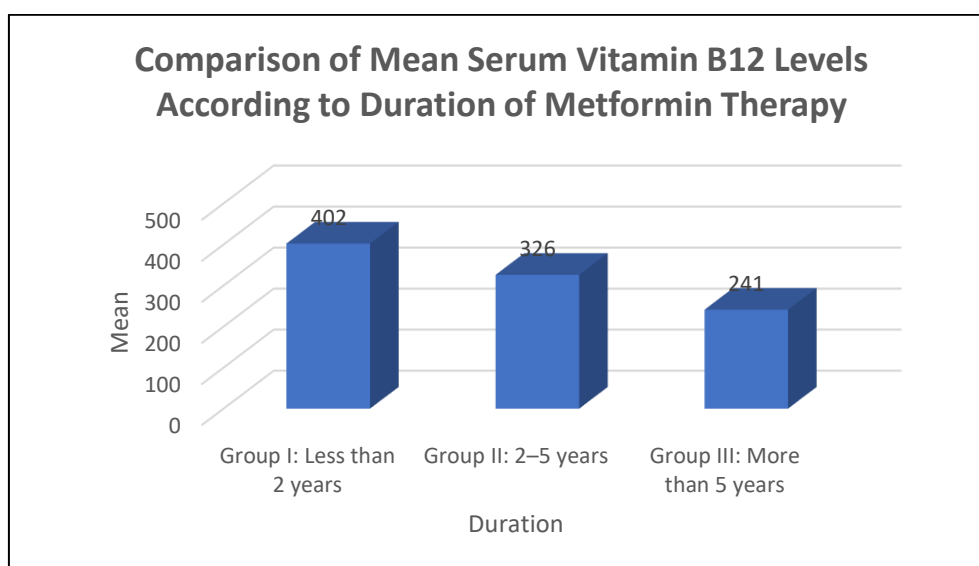


Figure 2. Comparison of Mean Serum Vitamin B12 Levels According to Duration of Metformin Therapy

Table 5. Association between Duration of Metformin Therapy and Serum Vitamin B12 Status

Vitamin B12 status	Group I (n=38)	Group II (n=46)	Group III (n=46)	p-value	Statistical test
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Deficient	3 (7.9%)	9 (19.6%)	19 (41.3%)	<0.001	Chi-square
Borderline	8 (21.1%)	15 (32.6%)	17 (37.0%)		
Normal	27 (71.1%)	22 (47.8%)	10 (21.7%)		
Total	38 (100%)	46 (100%)	46 (100%)		

Table 6. Correlation of Serum Vitamin B12 Levels with Duration of Metformin Therapy and Other Clinical Parameters (N=130)

Clinical parameter	Pearson's r	p-value	Statistical test
Duration of metformin therapy (years)	-0.52	<0.001	Pearson's correlation
Duration of diabetes mellitus (years)	-0.38	<0.001	
Daily metformin dosage (mg/day)	-0.31	<0.001	
Age (years)	-0.19	0.030	
BMI (kg/m ²)	-0.11	0.213	
HbA1c (%)	-0.22	0.012	

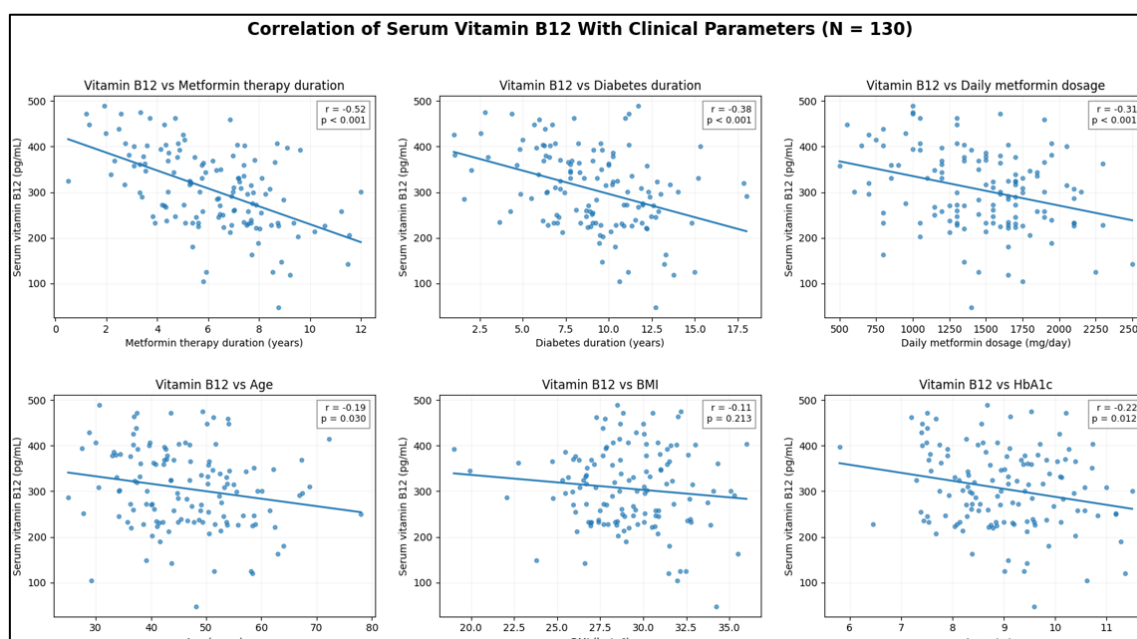


Figure 3. Correlation of Serum Vitamin B12 Levels with Duration of Metformin Therapy and Other Clinical Parameters (N=130)

DISCUSSION

The present study included 130 patients with T2DM, with a mean age of 54.6 ± 10.8 years and male predominance (56.2%). The mean duration of diabetes was 7.4 ± 4.2 years, mean BMI was 26.4 ± 3.8 kg/m², and mean daily metformin dosage was 1450 ± 486 mg. Herrmann and Obeid (2008) [13] identified advancing age, nutritional inadequacy and gastrointestinal disorders as important factors contributing to vitamin B12 deficiency, highlighting the need to consider these variables while assessing metformin-associated deficiency.

In the present study, 38 patients (29.2%) received metformin for less than two years, 46 (35.4%) for 2–5 years and 46 (35.4%) for more than five years. Prolonged exposure may progressively affect vitamin B12 absorption.

Herrmann and Obeid (2008) [13] emphasised that biochemical depletion may precede clinical manifestations, supporting vitamin B12 assessment among patients receiving long-term treatment.

Vitamin B12 deficiency was observed in 31 patients (23.8%), borderline concentrations in 40 (30.8%) and normal concentrations in 59 (45.4%). Thus, 54.6% had either deficient or borderline vitamin B12 levels.

Stabler et al. (1990) [14], in a prospective evaluation of 300 patients with serum vitamin B12 below 200 pg/mL, reported treatment responses in 86 patients, of whom 38% had initial concentrations between 100 and 199 pg/mL and 28% exhibited neuropsychiatric abnormalities. Elevated methylmalonic acid and/or homocysteine was observed in 94% of treatment-responsive patients. These findings,

together with the observations of Herrmann and Obeid (2008) [13], highlight the importance of further evaluation when vitamin B12 concentrations are borderline or clinical suspicion persists.

Mean serum vitamin B12 concentration progressively decreased from 402 ± 112 pg/mL in patients receiving metformin for less than two years to 326 ± 105 pg/mL in those receiving treatment for 2–5 years and 241 ± 96 pg/mL among patients receiving treatment for more than five years ($p < 0.001$).

Comparable findings were reported by de Jager et al. (2010) [11], who conducted a randomised trial involving 390 patients over 4.3 years and demonstrated a 19% reduction in vitamin B12 concentration with metformin compared with placebo. Vitamin B12 deficiency occurred in 9.9% of metformin-treated patients versus 2.7% of placebo recipients. Similarly, Aroda et al. (2016) [12] reported deficiency in 4.3% of metformin users compared with 2.3% of placebo recipients at five years ($p = 0.02$).

In the present study, vitamin B12 deficiency increased progressively across treatment-duration groups, from 7.9% in Group I to 19.6% in Group II and 41.3% in Group III ($p < 0.001$). Conversely, the proportion of patients with normal vitamin B12 levels declined from 71.1% to 47.8% and 21.7%, respectively.

These findings are consistent with Aroda et al. (2016) [12], who reported that each additional year of metformin exposure was associated with increased odds of vitamin B12 deficiency (adjusted OR: 1.13; 95% CI: 1.06–1.20). This supports the relevance of treatment duration when evaluating vitamin B12 status.

The correlation analysis demonstrated an inverse association between serum vitamin B12 concentration and duration of metformin therapy ($r = -0.52$; $p < 0.001$). Negative correlations were also proposed for duration of diabetes ($r = -0.38$), daily metformin dosage ($r = -0.31$), age ($r = -0.19$) and HbA1c ($r = -0.22$), whereas BMI showed a non-significant association ($r = -0.11$; $p = 0.213$).

Derosa et al. (2003) [15] compared metformin and repaglinide treatment over 12 months and reported improvements in glycaemic control and selected cardiovascular risk parameters. Although vitamin B12 was not directly evaluated, their findings provide context for the metabolic effects of metformin. The proposed correlations in the present study require confirmation using individual patient-level data.

Vitamin B12 deficiency is particularly relevant in T2DM because its neurological manifestations may overlap with diabetic peripheral neuropathy. The present study recorded a mean haemoglobin concentration of 12.6 ± 1.5 g/dL and mean corpuscular volume of 88.4 ± 7.6 fL; however, normal aggregate haematological values do not exclude deficiency in individual patients.

Healton et al. (1991) [16] evaluated 153 episodes of neurological cobalamin deficiency involving 143 patients and reported normal haematocrit in 27.4% of episodes and normal MCV in 23.0%. Similarly, Stabler et al. (1990) [14] documented neuropsychiatric abnormalities in 28% of treatment-responsive patients, frequently without anaemia or macrocytosis. These observations emphasise the importance of identifying vitamin B12 deficiency in patients presenting with neurological symptoms.

CONCLUSION

The present findings demonstrated a significant inverse association between duration of metformin therapy and serum vitamin B12 levels in patients with type 2 diabetes mellitus. The frequency of vitamin B12 deficiency increased progressively with prolonged treatment, particularly beyond five years. These findings highlight the potential importance of periodic vitamin B12 monitoring in patients receiving long-term metformin therapy.

Limitations

The single-centre, cross-sectional design and relatively small sample size limited the generalisability of findings and prevented establishment of a causal relationship. The absence of baseline vitamin B12 measurements and functional biomarkers, such as methylmalonic acid and homocysteine, may have affected the identification of early deficiency.

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