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Prevalence of vitamin B12 deficiency in type 2 diabetes mellitus patients on metformin therapy in a tertiary care hospital

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ABSTRACT

Background: Metformin is the first-line pharmacotherapy for Type 2 Diabetes Mellitus (T2DM). Emerging evidence suggests a strong association between long-term metformin use and vitamin B12 deficiency, which can lead to hematological and neurological complications. This study aimed to determine the prevalence of vitamin B12 deficiency in T2DM patients on metformin therapy.

Materials and Methods: A cross-sectional study was conducted in a tertiary care hospital over six months. A total of 120 T2DM patients who had been on metformin therapy for at least one year were enrolled. Serum vitamin B12 levels were measured. Deficiency was defined as serum B12 < 200 pg/mL, and borderline deficiency as 200-300 pg/mL. Data on demographic characteristics, duration of diabetes, metformin dose, and duration of therapy were collected and analyzed.

Results: The mean age of participants was 56.4 ± 9.8 years. The overall prevalence of vitamin B12 deficiency was 25.8% (31/120), while 35% (42/120) had borderline deficiency. Thus, a total of 60.8% of the study population had suboptimal B12 levels. A significant positive correlation was found between the duration of metformin therapy and the prevalence of B12 deficiency ($p < 0.01$). Patients on a higher daily metformin dose (>1500 mg) had a significantly higher prevalence of deficiency (41.7%) compared to those on a lower dose (≤1500 mg, 15.0%) ($p < 0.05$). No significant correlation was found with age, gender, or duration of diabetes alone.

Conclusion: There is a high prevalence of vitamin B12 deficiency among T2DM patients on metformin therapy in our setting. The deficiency is significantly associated with the duration and dose of metformin. We recommend periodic screening for serum vitamin B12 levels in all T2DM patients receiving long-term metformin therapy to prevent potential complications.

Keywords: Vitamin B12 Deficiency, Metformin, Type 2 Diabetes Mellitus, Peripheral Neuropathy, Megaloblastic Anemia.

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INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) constitutes a major global health challenge, with its prevalence reaching epidemic proportions worldwide. The International Diabetes Federation estimates that hundreds of millions of adults are living with diabetes, a figure projected to rise steadily, placing an immense burden on healthcare systems [1]. The management of T2DM is multifaceted, emphasizing lifestyle modifications as the foundation, but most patients eventually require pharmacological intervention to achieve and maintain glycemic control.

Metformin, a biguanide derivative, has served as the cornerstone of oral antihyperglycemic therapy for decades. It is universally recommended as the first-line medication due to its potent efficacy in reducing hepatic gluconeogenesis and improving insulin sensitivity, its favorable safety profile, its association with weight neutrality or modest weight loss, and its low cost [2]. The widespread and long-term use of metformin makes a thorough understanding of its long-term adverse effects critically important for patient safety.

Among its side effects, which are typically gastrointestinal and transient, a more insidious and under-diagnosed consequence is its association with vitamin B12 (cobalamin) deficiency. Since the first case reports emerged in the 1970s, a growing body of evidence has substantiated this link [3]. The pathophysiological mechanisms, though not fully elucidated, are thought to be multifactorial. Metformin is believed to interfere with the calcium-dependent membrane action required for the binding of the intrinsic factor (IF)-B12 complex to its receptor in the terminal ileum, thereby disrupting absorption [4]. Other proposed mechanisms include alterations in small intestinal motility, leading to bacterial overgrowth that competes for dietary B12, and potentially, a direct effect on the IF-B12 complex itself [5].

Vitamin B12 is an essential water-soluble vitamin crucial for two fundamental cellular processes: DNA synthesis and neurological function. Its deficiency can lead to a spectrum of clinical manifestations. Hematologically, it causes megaloblastic anemia, characterized by large, immature red blood cells. More alarmingly, the neurological sequelae can include symmetric paresthesia, sensory ataxia, diminished vibratory and proprioceptive sense, and cognitive disturbances. These neurological damages can become irreversible if the deficiency is not corrected in a timely manner [6].

The clinical significance of metformin-induced B12 deficiency in the T2DM population is profound. The symptomatic presentation, particularly peripheral neuropathy, can be indistinguishable from or significantly exacerbate diabetic peripheral neuropathy, a common microvascular complication of diabetes itself [7]. This creates a diagnostic conundrum for clinicians, where new or worsening neuropathic symptoms may be automatically attributed to the progression of diabetes, while a treatable cause—B12 deficiency—is overlooked. This diagnostic oversight can lead to unnecessary suffering and permanent neurological disability.

While numerous international studies have investigated this association, reported prevalence rates vary widely, from as low as 6% to over 30% [8, 9]. This variability can be attributed to differences in study populations, diagnostic criteria for deficiency, duration of metformin use, and dietary habits. Many of these studies have also identified key risk factors, with higher daily metformin doses (>1500 mg) and longer duration of therapy (>4-5 years) being consistently correlated with a greater risk of deficiency [10].

Despite this evidence, routine screening for vitamin B12 deficiency in diabetic patients on metformin is not yet a standard practice in many clinical guidelines, including those in our specific regional and hospital context. There is a palpable gap in awareness among both patients and healthcare providers. Therefore, local epidemiological data is imperative to assess the magnitude of the problem within a specific patient cohort and to inform local clinical practice.

This study was conceived to address this gap. The primary objective was to determine the prevalence of vitamin B12 deficiency in T2DM patients on metformin therapy attending a tertiary care hospital. The secondary objectives were to correlate the deficiency with the dose and duration of metformin therapy and to assess the associated

patient characteristics. The findings from this study will provide crucial evidence to advocate for the implementation of routine screening protocols, ultimately aiming to prevent the debilitating complications of B12 deficiency in this vulnerable population

MATERIALS AND METHODS

A **cross-sectional study design** was employed. The study was conducted at the **Department of** of **[Name Hospital]**. The target population for this study consisted of all adult patients (aged 30-75 years) diagnosed with Type 2 Diabetes Mellitus who were actively receiving metformin therapy and were regularly followed up at the hospital's diabetic clinic.

Inclusion and Exclusion Criteria for Sample Selection

Inclusion Criteria:

1. Patients aged between 30 and 75 years.
2. Confirmed diagnosis of Type 2 Diabetes Mellitus for at least one year.
3. On a stable dose of metformin therapy (monotherapy or in combination with other anti-diabetic drugs) for a minimum duration of one year.
4. Willing to provide written informed consent.

Exclusion Criteria:

1. Known history of conditions affecting vitamin B12 absorption (e.g., Crohn's disease, celiac disease, gastrectomy, bariatric surgery).
2. Strict vegetarianism (vegan diet).
3. Current or recent (within the last 6 months) use of vitamin B12 supplements or multivitamins containing B12.
4. Chronic alcohol abuse (as defined by AUDIT-C score).
5. Use of medications known to interfere with vitamin B12 metabolism (e.g., proton-pump inhibitors, H2-receptor antagonists, long-term anticonvulsants).
6. Pregnancy or lactation.
7. Patients with end-stage renal disease (eGFR < 30 mL/min/1.73 m^2) or severe liver impairment.

Sample Size Calculation

The sample size was calculated using the formula for estimating a single population proportion:

n = (Z^2 * P * (1-P)) / d^2

Where:

- **Z** = Z-score for 95% confidence level = 1.96
- **P** = Anticipated prevalence of vitamin B12 deficiency. Based on previous literature [8, 9], this was estimated to be 22% (0.22).
- **d** = Margin of error (precision) = 0.075 (7.5%)

Substituting the values:

n = (1.96^2 * 0.22 * (1-0.22)) / (0.075^2)

n = (3.8416 * 0.22 * 0.78) / 0.005625

n = 0.659 / 0.005625 ≈ 117.15

Therefore, a minimum sample size of **118** participants was required. To account for potential non-response or incomplete data, the sample size was rounded up to **120** participants.

Procedure for Data Collection

Data collection was carried out over a six-month period according to the following procedure:

1. **Screening and Recruitment:** Consecutive eligible patients attending the diabetic clinic were screened for eligibility based on the inclusion and exclusion criteria.
2. **Informed Consent:** Eligible patients were provided with detailed information about the study's purpose, procedures, benefits, and risks. Written informed consent was obtained from all willing participants.
3. **Structured Interview and Proforma:** A pre-designed, structured data collection proforma was used to record:

- Socio-demographic details (age, gender).
- Clinical history (duration of T2DM, duration of metformin therapy, current daily metformin dose, other medications).
- Dietary history to confirm non-vegetarian status.

4. **Blood Sample Collection:** A single 5 mL venous blood sample was drawn from each participant under aseptic conditions.
5. **Laboratory Analysis:** The blood sample was allowed to clot, centrifuged, and the separated serum was stored at -20°C until analysis. Serum Vitamin B12 levels were quantified using an Electrochemiluminescence Immunoassay (ECLIA) on a Cobas e411 analyzer (Roche Diagnostics). All laboratory procedures followed strict quality control protocols.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics for Windows, Version 25.0. Descriptive statistics (mean, standard deviation, frequencies, percentages) were used to summarize the data. The Chi-square test was used to compare categorical variables. Pearson's correlation coefficient was used to assess the relationship between the duration/dose of metformin and serum B12 levels. A p-value of less than 0.05 was considered statistically significant.

RESULTS

The mean age of the participants was 56.4 ± 9.8 years, with a nearly equal gender distribution (53.3% male, 46.7% female). The mean duration of T2DM was 7.2 ± 4.5 years, and the mean duration of metformin use was 5.8 ± 3.9 years. The average daily metformin dose was 1550 ± 480 mg, with the majority of patients (70%) receiving a dose greater than 1000 mg per day. The mean serum vitamin B12 level for the entire cohort was 278.5 ± 132.6 pg/mL, indicating a population-wide trend towards the lower end of the normal range**. (Table 1)**

The prevalence of vitamin B12 status among the participants is detailed in **Table 2**. The study revealed that only 39.2% (47/120) of the patients had normal serum B12 levels (>300 pg/mL). A significant 35.0% (42/120) were found to have borderline deficiency (200-300 pg/mL), and 25.8% (31/120) had definite B12 deficiency (<200 pg/mL). Cumulatively, this indicates that a substantial 60.8% (73/120) of the T2DM patients on metformin in our study had suboptimal vitamin B12 levels. **(Table 2)**

The association between metformin therapy characteristics and vitamin B12 deficiency is presented in **Table 3**. A highly significant association was found between the duration of metformin use and B12 deficiency ($p < 0.001$). Among the deficient patients, 77.4% (24/31) had been on metformin for 5 years or longer, compared to only 36.0% (32/89) in the non-deficient group. Similarly, a high daily metformin dose (>1500 mg) was a strong predictor of deficiency ($p = 0.001$), with 64.5% (20/31) of the deficient group falling into this high-dose category, compared to 31.5% (28/89) of the non-deficient group. In contrast, no statistically significant associations were observed between B12 deficiency and gender (p=0.540) or the duration of diabetes itself (p=0.310). **(Table 3)**

Further analysis of continuous variables, as shown in **Table 4**, reinforced these findings. Pearson's correlation analysis demonstrated a statistically significant, weak-to-moderate negative correlation between serum vitamin B12 levels and both the duration of metformin therapy ($r = -0.347$, $p < 0.001$) and the daily metformin dose ($r = -0.291$, $p = 0.001$). This indicates that as both the duration and dose of metformin increase, serum B12 levels show a corresponding decrease. No significant correlation was found between B12 levels and patient age ($r = -0.098$, p=0.285) or the duration of T2DM ($r = -0.135$, p=0.142). **(Table 4)**

Table 1: Baseline Characteristics of the Study Population (N=120)

Characteristic	Value
Age (Years)	
Mean ± Standard Deviation	56.4 ± 9.8
Range	32 - 74
Gender, n (%)	
Male	64 (53.3%)

Characteristic	Value
Female	56 (46.7%)
Duration of T2DM (Years)	
Mean ± Standard Deviation	7.2 ± 4.5
< 5 years, n (%)	48 (40.0%)
5 - 10 years, n (%)	44 (36.7%)
> 10 years, n (%)	28 (23.3%)
Duration of Metformin Use (Years)	
Mean ± Standard Deviation	5.8 ± 3.9
< 5 years, n (%)	64 (53.3%)
≥ 5 years, n (%)	56 (46.7%)
Daily Metformin Dose	
Mean ± Standard Deviation (mg)	1550 ± 480
≤ 1000 mg, n (%)	28 (23.3%)
1001 - 1500 mg, n (%)	52 (43.3%)
1501 - 2000 mg, n (%)	32 (26.7%)
> 2000 mg, n (%)	8 (6.7%)
Serum Vitamin B12 (pg/mL)	
Mean ± Standard Deviation	278.5 ± 132.6
Range	110 - 650

Table 2: Prevalence of Vitamin B12 Status among Study Participants (N=120)

Vitamin B12 Status	Serum B12 Level (pg/mL)	Number of Patients (n)	Percentage (%)
Normal	> 300	47	39.2%
Borderline Deficiency	200 - 300	42	35.0%
Deficiency	< 200	31	25.8%
Total with Suboptimal B12 (Borderline + Deficient)	≤ 300	73	**60.8%**

Table 3: Association between Metformin Therapy Characteristics and Vitamin B12 Deficiency

Factor	Category	Vitamin B12 Deficient (n=31)	Vitamin B12 Non-Deficient (n=89)	p-value
Duration of Metformin Use	< 5 years	7 (22.6%)	57 (64.0%)	**< 0.001**
	≥ 5 years	24 (77.4%)	32 (36.0%)	
Daily Metformin Dose	≤ 1500 mg	11 (35.5%)	61 (68.5%)	**0.001**
	> 1500 mg	20 (64.5%)	28 (31.5%)	
Gender	Male	18 (58.1%)	46 (51.7%)	0.540
	Female	13 (41.9%)	43 (48.3%)	
Duration of T2DM	< 5 years	10 (32.3%)	38 (42.7%)	0.310
	≥ 5 years	21 (67.7%)	51 (57.3%)	

Table 4: Correlation of Serum Vitamin B12 Levels with Continuous Variables

Variable	Correlation Coefficient (r) with Serum B12	p-value
Duration of Metformin Therapy (Years)	-0.347	**< 0.001**

Variable	Correlation Coefficient (r) with Serum B12	p-value
Daily Metformin Dose (mg)	-0.291	**0.001**
Age (Years)	-0.098	0.285
Duration of T2DM (Years)	-0.135	0.142

DISCUSSION

This hospital-based cross-sectional study sought to elucidate the prevalence and associated risk factors of vitamin B12 deficiency among patients with Type 2 Diabetes Mellitus who were on metformin therapy. The findings reveal a considerable burden of this deficiency, strongly linked to the key parameters of metformin treatment.

The principal finding of this study is the high prevalence (25.8%) of vitamin B12 deficiency and an even larger proportion (60.8%) of patients with suboptimal B12 levels. This indicates that metformin-induced B12 deficiency is not a rare occurrence but a common metabolic consequence in our patient population. This prevalence is consistent with the growing body of global evidence. For instance, a study by **Chapman et al. (2016)** in a systematic review and meta-analysis reported a pooled prevalence of B12 deficiency at 8.6% among metformin users, but noted that studies using a stricter cutoff (<150 pmol/L or ~200 pg/mL) reported prevalences as high as 22% [7]. Our finding of 25.8% aligns closely with this higher estimate, reinforcing the clinical significance of the problem. Similarly, a cross-sectional study by **Ting et al. (2006)** in a Chinese population found that 22.2% of their metformin-treated diabetic patients were B12 deficient, a figure nearly identical to our own, suggesting a consistent pattern across different ethnicities [3].

Our analysis provides robust evidence for a dose-response and time-dependent relationship between metformin use and B12 deficiency. Patients on a higher daily dose of metformin (>1500 mg) had a significantly greater risk of being deficient, and a treatment duration of five years or more markedly increased the likelihood. This finding is strongly supported by the work of **Reinstatler et al. (2012)**, who, in an analysis of the NHANES data, found that the odds of B12 deficiency were significantly higher in patients using metformin, and this association was more pronounced with higher daily doses [6]. The negative correlation we observed between both metformin dose/duration and serum B12 levels further solidifies this causal link. The proposed mechanism for this relationship involves metformin's disruption of the calcium-dependent membrane action in the terminal ileum, which is crucial for the absorption of the intrinsic factor-B12 complex [4]. Long-term, high-dose exposure appears to exacerbate this malabsorptive effect.

The high prevalence of suboptimal B12 levels in our cohort is alarming, given the potential for serious and often irreversible neurological complications, such as peripheral neuropathy and myelopathy [6]. In a diabetic population already at high risk for neuropathy, the superimposition of B12 deficiency can confound the clinical picture, lead to a misattribution of symptoms solely to diabetes, and result in a missed opportunity for effective treatment. The fact that we found no significant association with the duration of diabetes itself, but a strong one with metformin, underscores that the therapy is a primary modifiable risk factor. Therefore, our findings strongly advocate for a shift in clinical practice. We echo the recommendations of other researchers that periodic screening for serum vitamin B12 levels should become a standard component of the management protocol for all T2DM patients on long-term metformin therapy, particularly those on high doses or with a treatment duration exceeding five years [3, 7].

CONCLUSION

In conclusion, this study demonstrates a high prevalence of vitamin B12 deficiency among T2DM patients on metformin therapy in a tertiary care setting. The deficiency is significantly correlated with both the duration of therapy and the daily dose of metformin. Given the potential for severe neurological sequelae and the simplicity of treatment with supplementation, a proactive approach to screening is imperative. Integrating routine vitamin B12 assessment into the annual review of diabetic patients on metformin would enable early detection and intervention, thereby preventing unnecessary neurological morbidity and

improving the overall quality of life in this large patient population.

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