Prevalence and Risk Factors of Vitamin B12 Deficiency in Type 2 Diabetes Mellitus Patients on Metformin at a Tertiary Care Center of Nepal

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ABSTRACT

Background

Vitamin B12 deficiency is a common but often underrecognized complication in patients with type 2 diabetes mellitus (T2DM) undergoing long-term metformin therapy. If undetected, it may lead to serious complications including neuropathy, cognitive decline and anemia.

Objective

To determine the prevalence and risk factors of vitamin B12 deficiency in type 2 diabetes mellitus (T2DM) patients on metformin at a tertiary care center in Nepal.

Method

An analytical cross-sectional study was conducted among 318 patients with type 2 diabetes mellitus (T2DM) receiving metformin therapy. Serum vitamin B12 levels and relevant clinical and demographic data were collected. Descriptive statistics summarized baseline characteristics, while univariate and bivariate analyses were done. Multivariable logistic regression was then performed to identify independent predictors of vitamin B12 deficiency. Statistical significance was set at $p < 0.05$, and all analyses were conducted using Stata version 14.

Result

Vitamin B12 deficiency was observed in 21.7% (69 out of 318 participants). Multivariable regression analysis identified age (AOR: 1.07; 95% CI: 1.04 to 1.08; $p < 0.001$), metformin dose (AOR: 1.002; 95% CI: 1.001 to 1.003; $p < 0.001$) and longer metformin use (AOR: 1.11; 95% CI: 1.03 to 1.19; $p = 0.005$) were significantly associated with higher odds of deficiency. No significant associations were found with sex, smoking, alcohol use, vegetarian diet, HbA1c, lipid profiles, neuropathy, retinopathy or serum creatinine ($p > 0.05$).

Conclusion

Vitamin B12 deficiency is prevalent among metformin-treated T2DM patients, with age, duration and dose of metformin as key risk factors. Regular monitoring and supplementation are crucial for this patient population.

KEY WORDS

Metformin, Nepal, Type 2 diabetes mellitus, Vitamin B12 deficiency

INTRODUCTION

Metformin is widely used in the treatment of diabetes, particularly Type 2 Diabetes Mellitus (T2DM) and is often the first therapy prescribed.[1] It is known for its ability to increase insulin sensitivity and effectively lower blood sugar levels.[2] However, long-term use of metformin can hinder the absorption of Vitamin B12, which raises significant health concerns.[3]

Vitamin B12 is an essential nutrient and its deficiency can lead to various hematological and neurological disorders.[4,5] Previous studies have demonstrated a considerable prevalence of Vitamin B12 deficiency among T2DM patients who use metformin. For instance, research conducted by de Jager et al. indicated a 7.2 times higher prevalence of Vitamin B12 deficiency in metformin users compared to non-users.[1]

Diabetic patients experiencing a deficiency in Vitamin B12 may endure anemia, peripheral neuropathy, cognitive decline and cardiovascular issues. Vitamin B12 deficiency in diabetic patients can have serious implications and a poor prognosis if disregarded. However, this insufficiency is often neglected, despite many diabetic patients being at risk.[4,6] For example, the symptoms of diabetic neuropathy overlap with paresthesias, impaired sense of vibration and impaired proprioception associated with vitamin B12 deficiency. Consequently, nerve damage induced by vitamin B12 deficiency may be mistaken for or contribute to diabetic peripheral neuropathy. Identifying the correct cause of neuropathy is crucial, as straightforward Vitamin B12 supplementation can greatly improve the quality of life for patients by reversing neurological symptoms inappropriately attributed to hyperglycemia.[7] Moreover, Vitamin B12 replacement has demonstrated symptomatic improvement among patients with severe diabetic neuropathy.[8] This justifies that timely diagnosis and management of Vitamin B12 deficiency through regular monitoring and supplementation are vital to improving clinical outcomes and the quality of life for these patients.[9]

Despite its critical implications, there is limited data from South Asian countries, including Nepal, making it essential to investigate this issue within our specific demographic context. This highlights the need for further research to fill these knowledge gaps and improve the management of T2DM patients.[8] This study aims to establish the prevalence and identify the risk factors associated with Vitamin B12 deficiency among T2DM patients using metformin at a tertiary care center in Nepal. Understanding these factors will aid in developing strategies for early diagnosis and management of this deficiency.

METHODS

This analytical cross-sectional study was conducted at Dhulikhel Hospital, Kathmandu University School of Medical Sciences, Kavre, Nepal. The study was conducted over six months, from July to December 2023, after obtaining ethical approval from the Institutional Review Committee (Reference number: IRC-KUSMS 147/23). Written informed consent was obtained from all participants prior to enrollment.

The minimum sample size was calculated using the formula,

$n = Z^2 \times p \times q / e^2$

$= (1.96)^2 \times (0.247) \times (1-0.247) / 0.05^2$

$= 286$

where Z is the standard normal variate corresponding to a 95% confidence level (1.96), prevalence (p) was assumed to be 24.7% based on findings from a similar study by Tavares Bello et al., q = 1 – p and e is the margin of error (set at 5%).[10] We further added 10% to account for missing or incomplete data.

The final sample size was calculated by,

$n(final) = n / (1 - r) = 286 / (1-10\%) = 317.78$

Therefore, a total of 318 patients with T2DM on metformin were included in the study. Participants were selected using a simple random sampling method.

Patients with T2DM on metformin therapy for at least 6 months were included. Exclusion criteria included patients with known causes of vitamin B12 deficiency other than metformin use. Diagnosed with pernicious anemia, megaloblastic anemia of known etiology, history of bariatric or gastrointestinal surgery affecting absorption, chronic liver disease and chronic alcohol dependence.

Demographic and clinical information, including age, sex, dietary habits, smoking status, duration and dose of metformin therapy and relevant medical history, were collected through structured patient interviews and review of medical records. Blood samples were collected to measure serum vitamin B12 levels using the chemiluminescent microparticle immunoassay (CMIA) method. Vitamin B12 deficiency was defined as a serum level < 200 pg/mL. Additional laboratory investigations included HbA1c, lipid profile and serum creatinine levels.

The duration of T2DM was excluded from the multivariable regression analysis due to a near-perfect positive correlation (Pearson's $r = 0.999$) with the duration of metformin use, indicating multicollinearity (Fig. 1). Therefore, only the metformin duration was retained in the final model to avoid statistical redundancy.

All statistical analyses were performed using STATA version 14. Descriptive statistics (means, standard deviations, medians and proportions) were used to summarize demographic and clinical characteristics. We conducted bivariate and multivariable analyses to assess factors associated with vitamin B12 deficiency. A p-value < 0.05 was considered statistically significant.

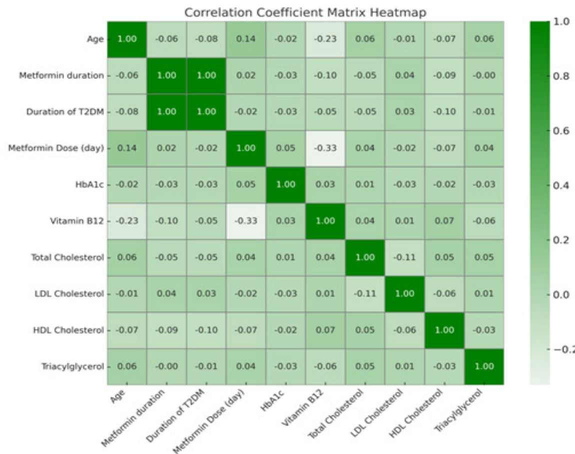

Figure 1. Correlation matrix of continuous study variables showing multicollinearity between T2DM duration and metformin duration

RESULTS

The study analyzed data from 318 patients with Type 2 Diabetes Mellitus (T2DM) on metformin at Dhulikhel Hospital, Nepal. The average age of participants was 57.2 ± 14.6 years, ranging from 28 to 83 years. The sample included 174 males and 144 females [Table 1].

Table 1. Baseline Demographic and Clinical Characteristics

Variable	Mean ± SD
Age (years)	57.2 ± 14.6
Duration of T2DM (years)	8.8 ± 4.6
Metformin duration (years)	9.0 ± 4.6
Metformin dose (mg/day)	1682 ± 759
HbA_1c (%)	9.8 ± 2.7
Serum vitamin B_{12} (pg/mL)	396 ± 202

The residences of participants varied across several regions, with the majority from Kavre 213(66.98%), followed by Bhaktapur 74(23.27%), Ramechhap 16(5.03%), Sindhupalchowk 12(3.77%) and Dolakha 3(0.94%) [Table 2].

Table 2. Geographical Distribution of Study Participants by Residence (n = 318)

Address	n (%)
Kavre	213 (66.98)
Bhaktapur	74 (23.27)
Ramechhap	16 (5.03)
Sindhupalchowk	12 (3.77)
Dolakha	3 (0.94)

Vitamin B12 levels ranged from 55 pg/mL to 749 pg/mL, with a median of 376 pg/mL. Vitamin B12 deficiency (< 200 pg/mL) was observed in 69 participants (21.7%), with 41 males and 28 females affected [Fig. 2]. The average duration of metformin use was 8.9 ± 4.6 years, ranging from 6 months to 17.6 years (including the years of intake of metformin

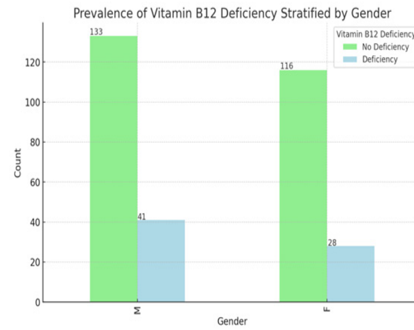

Figure 2. Distribution of study participants and vitamin B12 deficiency according to sex

from the prediabetic phase) and the average duration of T2DM was 8.8 ± 4.6 years, ranging from 6 months to 16.9 years. The mean daily metformin dose was 1682 ± 759.63 mg/day, ranging from 500 to 3000 mg. Vitamin B12 levels tended to decline with increasing metformin dosage [Fig. 3]. The prevalence of vitamin deficiency was higher in the age group of more than 60 years [Table 3]. Vitamin B12 levels tended to be lower in older age groups [Fig. 4].

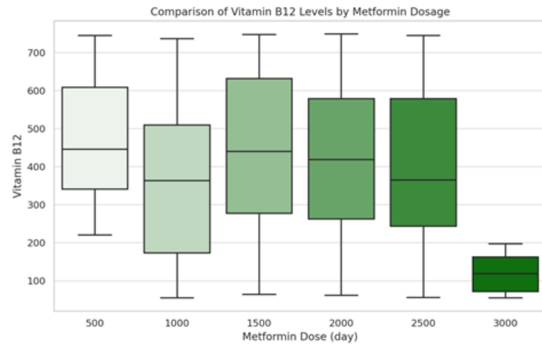

Figure 3. Box Plot diagram of comparison of vitamin B12 levels by metformin dosage

Table 3. Distribution of Vitamin B12 Deficiency Among T2DM Patients on Metformin by age group (n = 318)

Age	Vitamin B12 Deficiency n (%)
< 40 Years	0 (0.00%)
40-50 Years	2 (4.44%)
51-60 Years	20 (28.99%)
> 60 Years	47 (68.11%)

Percentages are calculated among vitamin B12 deficient participants (n=69).

Among the participants, 176 (55.35%) had diabetic neuropathy, and 161(50.63%) had retinopathy 174(54.72%) participants reported alcohol consumption, 41(12.89%) followed a vegetarian diet, 28(8.8%) reported intake of vitamin supplements and 141(44.34%) were smokers [Table 4].

The bivariate analyses confirmed significant relationships between vitamin B12 deficiency and various variables. In the crude logistic regression analysis, increasing age was significantly associated with higher odds of vitamin B12

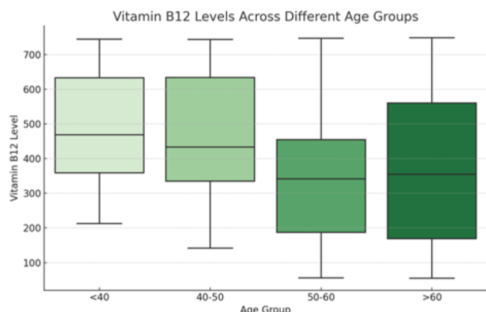

Figure 4. **Box Plot diagram of vitamin B12 levels across different age groups**

Table 4. **Clinical Characteristics and Lifestyle Factors of Study Participants (n = 318)**

Variable	Yes	No
Neuropathy	176 (55.3%)	142 (44.7%)
Retinopathy	161 (50.6%)	157 (49.4%)
Alcohol Consumption	174 (54.7%)	144 (45.3%)
Vegetarian	41 (12.8%)	277 (87.1%)
Smoking	141 (44.3%)	177 (55.7%)
Intake of Vitamin Supplements	28 (8.8%)	290 (91.1%)

deficiency (OR: 1.06; 95% CI: 1.035 to 1.082; p < 0.001). Duration of metformin use also showed a significant association (OR: 1.07; 95% CI: 1.00 to 1.12; p = 0.037). Similarly, a higher daily metformin dose was associated with increased odds of deficiency (OR: 1.0019, p < 0.001).

In the multivariable logistic regression analysis, after adjusting for age, sex, metformin dose and metformin duration, the above associations remained independently associated with vitamin B12 deficiency. Each additional year of age increased the odds of deficiency by 7% (AOR: 1.07; 95% CI: 1.04–1.08; p < 0.001). Duration of metformin use also remained a significant predictor, with each additional year of use increasing the odds by 11% (AOR: 1.11; 95% CI: 1.03 to 1.19; p = 0.005). Furthermore, higher daily doses of metformin remained significantly associated with increased odds of vitamin B12 deficiency (AOR: 1.002; 95% CI: 1.001–1.003; p < 0.001). All other variables assessed, including demographic, clinical and biochemical factors were not significantly associated with vitamin B12 deficiency in the bivariate or adjusted multivariable model [Table 5].

Table 5. **Multivariable Logistic Regression Analysis of Risk Factors for Vitamin B12 Deficiency in T2DM Patients on Metformin (n = 318)**

Characteristics	Bivariable			Multivariable*		
	Odds Ratio	95% CI	P-value	Odds Ratio	95% CI	P-value
Age	1.06	(1.04 to 1.08)	<0.001	1.07	(1.04 to 1.08)	**<0.001**
Gender						
Female	Ref					
Male	1.27	(0.74 to 2.19)	0.376	1.19	(0.61 to 2.33)	0.608
Metformin Duration	1.07	(1.00 to 1.12)	**0.037**	1.11	(1.03 to 1.19)	**0.005**
Metformin dose/Day	1.001	(1.001 to 1.002)	**0.001**	1.002	(1.001 to 1.003)	**0.001**
Address						
Kavre	Ref					
Bhaktapur	1.09	(0.57 to 2.08)	0.793	1.12	(0.51 to 2.43)	0.778
Ramech-hap	2.37	(0.81 to 6.89)	0.112	1.06	(0.27 to 4.16)	0.938
Sindhupal-chowk	1.32	(0.34 to 5.08)	0.688	0.65	(0.17 to 4.81)	0.672
Dolakha	1.98	(0.18 to 22.31)	0.582	1.49	(0.10 to 22.01)	0.771
Symptoms: Neuropathy						
No	Ref					
Yes	0.99	(0.57 to 1.69)	0.959	1.34	(0.68 to 2.63)	0.392
Retinopathy						
No	Ref					
Yes	0.80	(0.47 to 1.37)	0.425	0.97	(0.51 to 1.92)	0.970
Alcohol						
No	Ref					
Yes	1.01	(0.59 to 1.74)	0.947	1.00	(0.51 to 1.96)	0.982
Vegetarian						
No	Ref					
Yes	1.38	(0.66 to 2.92)	0.395	0.92	(0.34 to 2.45)	0.864
Smoking						
No	Ref					
Yes	1.38	(0.81 to 2.37)	0.5	1.17	(0.61 to 2.28)	0.636
Blood Investigation						
HbA1c	0.98	(0.89 to 1.08)	0.739	0.95	(0.84 to 1.07)	0.457
Total Cho-lesterol	1.00	(0.99 to 1.00)	0.633	0.99	(0.99 to 1.01)	0.938
HDL Cho-lesterol	0.99	(0.97 to 1.01)	0.626	1.01	(0.98 to 1.03)	0.466
LDL Cho-lesterol	1.00	(0.99 to 1.00)	0.467	1.00	(0.99 to 1.01)	0.351
Triacylg-lycerol	1.00	(0.99 to 1.01)	0.06	1.00	0.99 to 1.01	0.121
Serum Creatinine	1.07	(0.93 to 1.23)	0.798	1.41	(0.72 to 2.76)	0.320

*Adjusted for Age, Sex, Metformin dose and Metformin duration

DISCUSSIONS

This study explores the prevalence and risk factors of vitamin B12 deficiency among Type 2 diabetes (T2DM) patients undergoing metformin therapy at a tertiary care center in Nepal.

The prevalence of vitamin B12 deficiency among type 2 diabetes mellitus (T2DM) patients using metformin varies significantly in different studies. Globally, studies have reported that a significant proportion, ranging from 6% to 30% of patients, could potentially experience vitamin B12 deficiency due to metformin use.[11,12]

Our study found a prevalence of 21.7%, which is consistent with several previous studies. For example, Almatrafi et al. found a 17.5% prevalence in Saudi Arabia and Kim et al. reported a 22.2% prevalence in studies in Korea.[13,14] Additionally, Rathis et al. found a prevalence of 27%, which falls within a comparable range.[15]

However, our research findings are quite different from those of studies conducted in Nepal. Sah et al. found a much higher prevalence of 78%, while Malla et al. reported a prevalence of 50.95%.[16,17] Almatrafi et al. suggested that cultural and dietary differences, as well as varying cutoff values for defining vitamin B12 deficiency, contribute to these inconsistencies.[13] Our prevalence values are similar to global data but contrast sharply with studies conducted in Nepal, highlighting the need for region-specific assessments.

Despite the variations in prevalence percentages, it is clear that there is a significant occurrence of vitamin B12 deficiency among patients undergoing metformin therapy. This emphasizes the need for regular monitoring and appropriate management of vitamin B12 levels in these patients to prevent potential complications associated with deficiency.

In our study, we observed a higher prevalence of vitamin B12 deficiency among elderly individuals undergoing metformin therapy, 28.99% (n=20) in 51-60 years and 68.11% (n=47) in participants more than 60 years [Table 3]. This could be attributed to the longer duration of medication use in the elderly, or age itself serving as a risk factor for vitamin B12 deficiency. This finding was further supported by our multivariable logistic regression analysis, which showed that after adjusting for sex, metformin dose and duration of metformin use, each additional year of age increased the odds of deficiency by 7% (AOR: 1.07; 95% CI: 1.04–1.08; $p < 0.001$) [Table 5].

Our findings are consistent with those of Sah et al. who noted higher deficiency rates in the 50-60 age group and with Allen et al. who reported that the prevalence of vitamin B12 deficiency increases with age, with approximately 6% of those aged 60 years and older being deficient and nearly 20% having marginal status.[16,18] Allen suggests that food-bound cobalamin malabsorption, which increases with age due to gastric atrophy and reduced gastric acid secretion, is a significant cause of this deficiency.[18]

Furthermore, our study observed a numerically higher prevalence of vitamin B12 deficiency among males than females, although this difference was not statistically significant [Fig. 2]. This aligns with the findings of Margalit et al. who discovered higher deficiency rates in men (25.5%) compared to women (18.9%) and hypothesized that genetic variations rather than diet or estrogen effects might contribute to this difference, which could also explain our findings.[19]

It's concerning to note that despite the American Diabetes Association's recommendation of a daily metformin dose of 500 mg to 2550 mg, our study revealed that 16 patients were taking 3000 mg per day. This likely stems from a lack of appropriate drug counseling, particularly for individuals with low literacy levels.[20]

Also, our study found a connection between longer duration and higher doses of metformin and a greater risk of vitamin B12 deficiency, as supported by both crude and adjusted logistic regression analyses. Specifically, each year of metformin use increased the odds of deficiency by 7% in crude analysis and by 11% in the adjusted model, while higher metformin dose was also independently associated with increased odds in both models [Table 1]. This is consistent with the findings of Sah et al. and Malla et al.[16,17] Specifically, Sah et al. reported a 94% deficiency rate among those taking more than 1000 mg per day, while only 39% of people with diabetes taking less than 1000 mg per day were found to be deficient.[16] Additionally, Almatrafi et al. found that 97.2% of patients with a deficiency were using metformin for more than 2 years, although they did not find a significant association between dose and deficiency.[13] However, Rathis et al. confirmed that both longer duration and higher doses are significant risk factors.[15]

The mechanism behind this association can be explained by metformin's interference with the absorption of vitamin B12 in the small intestine. Metformin affects the calcium-dependent membrane action responsible for vitamin B12 absorption in the ileum, as reported by Sayedali et al.[21] Prolonged use and higher doses of metformin can exacerbate this interference, significantly reducing vitamin B12 levels over time. This explanation is crucial for understanding why patients on long-term metformin therapy, particularly at higher doses, are at increased risk for vitamin B12 deficiency.

While our study included data on vitamin B12 supplementation, our regression did not find vitamin B12 supplement use to be a significant predictor of deficiency, likely because only 28 (8.8%) of participants

used supplements, limiting statistical power [Table 1]. The Framingham Offspring Study showed that people who consumed higher amounts of vitamin B12 from all sources, including supplements and fortified cereals, had higher levels of serum vitamin B12.[22] Specifically, plasma vitamin B12 levels increased by 45 pmol/L for each doubling of intake, with similar benefits from supplements and fortified cereals compared to other foods.[22] Malla et al. have recommended annual screening and supplementation for long-term metformin users to reduce the risk of vitamin B12 deficiency.[17] Additionally, Shaimaa et al. found that low dietary intake increased the risk of vitamin B12 deficiency by approximately 44.[13]

Although strict vegetarianism is a known risk factor for B12 deficiency due to the lack of animal-source foods, we found no significant association in our study (p=0.864).[23] This may be because our study lacked the power to detect such an effect for a small number of vegetarians 41(12.81%). Similarly, alcohol consumption (p = 0.982) and smoking (p = 0.636) were not significantly associated with deficiency [Table 4 and 5].

Diabetic neuropathy and retinopathy were highly prevalent in our sample (55% and 51%, respectively), but neither showed a significant association with vitamin B12 deficiency (p = 0.392 and p = 0.970) [Table 4 and 5]. These findings suggest that microvascular complications in T2DM may occur independently of B12 status or that B12-deficient neuropathy may be clinically misattributed to diabetes. Our result aligns with a large Kuwaiti study, which also reported no association between B12 deficiency and diabetic neuropathy.[24] In contrast, a recent meta-analysis by Yang et al. reported significantly lower B12 levels in patients with diabetic retinopathy, highlighting possible population or methodological differences that indicates further study.[25] We also observed no association with glycemic control (HbA1c), serum creatinine or lipid profiles. These results are consistent with findings from Al-Hamdi et al. in Oman, who similarly found no correlation between B12 levels and HbA1c, hemoglobin, age, sex, or duration of diabetes.[26]

Based on our study, we have found that regular monitoring of vitamin B12 levels is essential for patients with type 2 diabetes mellitus (T2DM) who are taking metformin. This is important to prevent complications like neuropathy, cognitive decline, anemia and cardiovascular issues. We recommend annual screening and appropriate supplementation, especially for those on high doses or long-term therapy. Clinicians should be watchful and proactive in managing vitamin B12 levels to improve patient outcomes However, measuring B12 directly can detect subclinical deficiency before overt anemia develops. The exclusion of T2DM duration from our model was necessary due to its near-perfect correlation with metformin duration (r=0.999), indicating these variables measure virtually identical exposure timeframes in this cohort [Fig. 1].

However, it is important to note that our study's cross-sectional design limits the ability to draw causal conclusions. Future studies should use longitudinal designs and larger sample sizes to validate these findings.

We did not incorporate hematological parameters such as complete blood counts or peripheral blood smear findings into the present analysis, as these data are being evaluated separately in an ancillary study. This is a limitation of the current study, as hematological abnormalities may provide additional evidence of vitamin B12 deficiency.[23] Nevertheless, direct measurement of serum vitamin B12 levels allowed identification of deficiency even before overt hematological manifestations developed.[4]

Additionally, a more comprehensive approach to assessing clinical neurological symptoms experienced by patients would offer valuable insights and help in making necessary adjustments to treatment protocols. It's also important to consider the impact of other medications, such as proton pump inhibitors (PPIs) and H2-receptor antagonists, known to lower vitamin B12 levels, which were not accounted for in our study. Addressing these factors in future research could further clarify the relationship between vitamin B12 deficiency and its management.

CONCLUSION

Our study demonstrated a significant prevalence of vitamin B12 deficiency among T2DM patients on metformin, highlighting the impact of increasing age, longer metformin use and higher doses as substantial risk factors. Strengths of this study include a larger sample size and standard laboratory measures, although the cross-sectional design limits causal inferences. Multivariable logistic regression analysis was employed to control for potential confounders, ensuring a more accurate interpretation of the data. The findings align with global literature but reveal discrepancies with local studies, emphasizing the need for localized assessments. Future research should employ longitudinal designs to confirm these associations and explore the role of other medications affecting vitamin B12 levels. We recommend regular monitoring and supplementation for T2DM patients on metformin to mitigate the risk of deficiency and its associated complications, thereby enhancing patient management and outcomes.

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