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Frequency of Vitamin B12 Deficiency Anemia in Diabetic Patients on Metformin in a Tertiary Care Hospital, Lahore, Pakistan

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ABSTRACT

Objective: To determine the frequency of vitamin B12 deficiency in patients with type 2 diabetes mellitus receiving metformin at a tertiary care hospital in Lahore, Pakistan, and to assess its association with age, gender, duration of diabetes, duration of metformin use, and body mass index. **Methodology:** This cross-sectional study was conducted in the Outpatient Department of Internal Medicine, Gulab Devi Hospital, Lahore, over six months (December 2024 to May 2025). A total of 150 patients aged 18–70 years with type 2 diabetes mellitus on metformin monotherapy for at least six months were included through non-probability consecutive sampling. Pregnant and lactating women, vegans, and patients with diagnosed diabetic peripheral neuropathy were excluded. Serum vitamin B12 level was measured in all participants, and deficiency was defined as a level below 150 pg/ml. Data were analyzed using SPSS version 25. Quantitative variables were expressed as mean ± standard deviation, while categorical variables were presented as frequency and percentage. Independent samples t-test and chi-square test were applied, and p-value <0.05 was considered significant. **Results:** The mean age of the patients was 51.8 ± 9.6 years, and 56.0% were females. The mean duration of diabetes was 6.8 ± 3.1 years, while the mean duration of metformin use was 5.6 ± 2.5 years. Mean body mass index was 28.4 ± 4.3 kg/m^2, and mean serum vitamin B12 level was 214.9 ± 86.3 pg/ml. Vitamin B12 deficiency was found in 47 patients, giving a frequency of 31.3%. Deficiency showed significant association with increasing age (p=0.031), female gender (p=0.028), longer duration of diabetes (p=0.021), and longer duration of metformin use (p<0.001). No significant association was found with body mass index category (p=0.212). **Conclusion:** Vitamin B12 deficiency was common in metformin-treated patients with type 2 diabetes mellitus and was significantly associated with increasing age, female gender, longer duration of diabetes, and prolonged metformin use.

INTRODUCTION

Type 2 diabetes mellitus is a major global health problem and its burden continues to rise, particularly in middle- and low-income countries [1,2]. In Pakistan, its reported prevalence ranges from 7.2% to 19.21% [3]. Metformin remains the drug of choice in most patients with type 2 diabetes mellitus, while alternative or second-line treatment options are selected according to individual patient characteristics [4]. Although the most common adverse effects of metformin are usually mild, transient, and gastrointestinal in nature [5], one clinically important but often under-recognized complication is vitamin B12 malabsorption [6].

Diabetes mellitus is associated with long-term complications involving the kidneys, eyes, blood vessels, heart, and nerves [7,8]. Metformin is the most frequently prescribed oral hypoglycemic agent and is recommended as first-line therapy by both the European Association for the Study of Diabetes and the American Diabetes Association. However, prolonged metformin use has been linked with vitamin B12 deficiency, which may remain clinically silent and therefore requires close monitoring. Previous studies have shown that serum vitamin B12 levels are inversely related to both the dose and duration of metformin therapy [9]. It has also been reported that 10% to 30% of diabetic patients may develop some degree of vitamin B12 malabsorption. Although the strength of the association between metformin uses and vitamin B12 deficiency has remained debated, screening and monitoring of vitamin B12 levels have been advised in patients with type 2 diabetes mellitus, particularly in those receiving long-term metformin treatment [10].

Vitamin B12 deficiency may lead to altered mental status, megaloblastic anemia, and neurodegeneration. In diabetic patients, the clinical picture may become more complex because manifestations such as paresthesia, impaired

vibration sense, and loss of proprioception may overlap with features of diabetic neuropathy [11]. Several other factors have also been implicated in vitamin B12 deficiency, including ageing, genetic disorders affecting bone health, physical inactivity, estrogen deficiency in postmenopausal women, and dietary factors, the latter being particularly important because they are potentially modifiable [12].

Furthermore, pernicious anemia has also been associated with an increased risk of fractures. The proposed mechanisms of vitamin B12 deficiency in metformin-treated patients include altered bowel motility with bacterial overgrowth, leading to impaired absorption of vitamin B12. In addition, metformin interferes with calcium-dependent absorption of the vitamin B12–intrinsic factor complex in the terminal ileum, thereby reducing vitamin B12 absorption. This effect has been reported to improve with calcium supplementation [6,13].

Local and regional studies have also demonstrated a considerable burden of vitamin B12 deficiency among diabetic patients receiving metformin. A study reported that serum vitamin B12 levels were significantly higher in non-diabetic controls than in metformin-treated diabetic patients (301.71 ± 72.12 vs 189.25 ± 31.22; p<0.0001). Vitamin B12 deficiency was also more frequent in the diabetic group (39% vs 12%; p<0.0001), and serum vitamin B12 levels declined significantly with increasing duration of metformin use (p<0.0001) [1]. In another cross-sectional study in 2022 among 240 patients with type 2 diabetes mellitus, the mean vitamin B12 level was 240 ± 76.6 pg/ml. Normal vitamin B12 levels above 300 pg/ml were found in 41 (17.1%) patients, borderline levels in 97 (40.4%), and deficiency in 102 (42.5%). A significant gender-based association was also reported [7]. Similarly, a cross-sectional study reported vitamin B12 deficiency in 25% of patients with type 2 diabetes mellitus receiving metformin therapy [14]. A Brazilian study further showed that the frequency of vitamin B12 deficiency in patients receiving prolonged metformin treatment ranged from 5.8% to 30% [6].

In view of the above findings and the need to reduce morbidity among adults with diabetes, the occurrence of vitamin B12 deficiency related to metformin use requires focused evaluation in Lahore. Therefore, this study was planned to determine the frequency of vitamin B12 deficiency anemia in diabetic patients receiving metformin at a tertiary care hospital in Lahore, Pakistan.

MATERIALS AND METHODS

This cross-sectional study was conducted in the Outpatient Department of Internal Medicine, Gulab Devi Hospital, Lahore, over a period of six months (December 2024 to May 2025). Ethical approval was taken. A sample size of 150 patients was calculated using a 95% confidence interval, an anticipated proportion of 25% for vitamin B12 deficiency among patients with type 2 diabetes mellitus, and a margin of error of 7%. Patients were recruited by non-probability consecutive sampling. The study included adult patients of both genders aged 18 to 70 years who had type 2 diabetes mellitus diagnosed at least six months earlier and were receiving metformin monotherapy for at least six months. Patients who were pregnant or lactating, those following a vegan diet, and those with diagnosed diabetic peripheral neuropathy were excluded.

For the purpose of the study, vitamin B12 deficiency was defined as a serum vitamin B12 level of less than 150 pg/ml. Body mass index was calculated by dividing body weight in kilograms by the square of height in meters and was categorized according to Asian criteria as normal (18.5 kg/m^2 to <23 kg/m^2), overweight (23 kg/m^2 to <27.5 kg/m^2), and obese (≥27.5 kg/m^2). Adult population was defined as patients aged 18 to 70 years. Diabetic patients were considered eligible if the diagnosis had been established at least six months earlier and metformin had been used for at least six months.

Data were collected from 150 eligible patients using a structured proforma. Information regarding demographic characteristics and clinical variables, including duration of diabetes and duration of metformin use, was recorded. After verbal informed consent had been obtained, each participant underwent anthropometric assessment, including measurement of weight and height, for calculation of body mass index. Under aseptic conditions, 2 mL of venous blood was drawn and sent to the Gulab Devi Hospital laboratory for measurement of serum vitamin B12 level. All findings were recorded on the study proforma, and vitamin B12 deficiency was documented according to the predefined operational definition.

Data were entered and analyzed using Statistical Package for the Social Sciences version 25. Quantitative variables were expressed as mean ± standard deviation, whereas categorical variables were presented as frequencies and percentages. Chi-square test and independent samples t-test were applied, as appropriate, to assess the association of vitamin B12 deficiency with age, gender, duration of disease, duration of metformin use, and body mass index. Effect modifiers, including age and gender, were controlled through stratification. A p-value of less than 0.05 was considered statistically significant.

RESULTS

A total of 150 patients with type 2 diabetes mellitus receiving metformin monotherapy were included in the study. The mean age was 51.8 ± 9.6 years. Females constituted 56.0% of the study population, while 44.0% were males. The mean duration of diabetes was 6.8 ± 3.1 years, and the mean duration of metformin use was 5.6 ± 2.5 years. The mean body mass index was 28.4 ± 4.3 kg/m^2, with obesity being the most frequent body mass index category. The mean serum vitamin B12 level of the overall study population was 214.9 ± 86.3 pg/mL.

Table 1

Baseline Demographic and Clinical Characteristics of the Study Population (n = 150)

Variable	Value
Age (years), mean ± SD	51.8 ± 9.6
Gender, male, n (%)	66 (44.0)
Gender, female, n (%)	84 (56.0)
Duration of diabetes (years), mean ± SD	6.8 ± 3.1
Duration of metformin use (years), mean ± SD	5.6 ± 2.5
Body mass index (kg/m^2), mean ± SD	28.4 ± 4.3
Serum vitamin B12 (pg/mL), mean ± SD	214.9 ± 86.3
Age 18–40 years, n (%)	24 (16.0)
Age 41–55 years, n (%)	71 (47.3)

Age 56–70 years, n (%)	55 (36.7)
Duration of diabetes ≤5 years, n (%)	63 (42.0)
Duration of diabetes 6–10 years, n (%)	61 (40.7)
Duration of diabetes >10 years, n (%)	26 (17.3)
Metformin use 6–12 months, n (%)	22 (14.7)
Metformin use >1–3 years, n (%)	44 (29.3)
Metformin use >3 years, n (%)	84 (56.0)
Normal body mass index, n (%)	28 (18.7)
Overweight, n (%)	54 (36.0)
Obese, n (%)	68 (45.3)

Table 2

Frequency of Vitamin B12 Deficiency in Metformin-Treated Patients (n = 150)

Vitamin B12 status	n	%
Deficient (<150 pg/mL)	47	31.3
Not deficient (≥150 pg/mL)	103	68.7

On stratified analysis, vitamin B12 deficiency was significantly associated with female gender, higher age category, longer duration of diabetes, and longer duration of metformin use. No significant association was observed between body mass index category and vitamin B12 deficiency. These findings are consistent with the available regional literature, which has shown a substantial burden of vitamin B12 deficiency among metformin-treated patients with type 2 diabetes mellitus and a stronger relationship with duration of treatment than with anthropometric parameters.

Table 3

Comparison of Quantitative Variables Between Vitamin B12 Deficient and Non-Deficient Patients

Variable	Vitamin B12 deficient (n=47)	Not deficient (n=103)	Test value (t)	p-value
Age (years), mean ± SD	55.4 ± 9.2	50.2 ± 9.4	3.19	0.002
Duration of diabetes (years), mean ± SD	8.1 ± 3.3	6.2 ± 2.8	3.42	0.001
Duration of metformin use (years), mean ± SD	6.8 ± 2.6	5.1 ± 2.4	3.80	<0.001
Body mass index (kg/m^2), mean ± SD	29.1 ± 4.5	28.1 ± 4.2	1.29	0.201
Serum vitamin B12 (pg/mL), mean ± SD	124.8 ± 16.9	256.0 ± 64.5	—	—

Table 4

Association of Categorical Variables with Vitamin B12 Deficiency

Variable	Vitamin B12 deficient n (%)	Not deficient n (%)	Chi-square	p-value
Gender			4.80	0.028
Male (n=66)	14 (21.2)	52 (78.8)		
Female (n=84)	33 (39.3)	51 (60.7)		
Age group			6.96	0.031
18–40 years (n=24)	4 (16.7)	20 (83.3)		
41–55 years (n=71)	19 (26.8)	52 (73.2)		
56–70 years (n=55)	24 (43.6)	31 (56.4)		
Duration of diabetes			7.69	0.021
≤5 years (n=63)	12 (19.0)	51 (81.0)		
6–10 years (n=61)	24 (39.3)	37 (60.7)		
>10 years (n=26)	11 (42.3)	15 (57.7)		
Duration of metformin use			14.91	<0.001
6–12 months (n=22)	2 (9.1)	20 (90.9)		
>1–3 years (n=44)	8 (18.2)	36 (81.8)		
>3 years (n=84)	37 (44.0)	47 (56.0)		
Body mass index category			3.10	0.212
Normal (n=28)	6 (21.4)	22 (78.6)		
Overweight (n=54)	15 (27.8)	39 (72.2)		
Obese (n=68)	26 (38.2)	42 (61.8)		

DISCUSSION

The present study found that vitamin B12 deficiency was present in 31.3% of patients with type 2 diabetes mellitus receiving metformin. This frequency is clinically important and is broadly consistent with previous literature showing that metformin-associated vitamin B12 deficiency is common, although reported prevalence varies substantially according to study population, duration of therapy, dose of metformin, and cut-off used to define deficiency [13–16]. In the current series, the observed frequency was lower than that reported in some recent studies, including the Peshawar study by Khan et al. and the Nigerian study by Owhin et al., but remained within the wider range described in systematic reviews and meta-analyses [14,16,20–22]. These findings support the view that vitamin B12 deficiency is not a rare adverse effect of metformin therapy and deserves routine clinical attention.

An important finding of the present study was the significant association of vitamin B12 deficiency with increasing age, longer duration of diabetes, and longer duration of metformin use. Among these, duration of metformin exposure appeared to be the strongest correlate. This is in line with previous work by Ting et al., who showed that both dose and duration of metformin were independently associated with vitamin B12 deficiency [17]. Similar observations were reported by Aroda et al. in the Diabetes Prevention Program Outcomes Study and by Reinstatler et al. in a population-based analysis, both of which demonstrated a higher frequency of biochemical deficiency with prolonged metformin use [18,19]. More recent reviews and cross-sectional studies have reached similar conclusions and have reinforced the importance of cumulative exposure as a major determinant of deficiency [16,21,22].

The association observed with female gender in the present study also merits attention. Although gender-based findings have not been uniform across all published studies, some regional reports have similarly noted a higher burden of low vitamin B12 levels among women [20]. This may reflect differences in nutritional status, body stores, dietary intake, and health-seeking behavior rather than a direct biological effect alone. In contrast, body mass index category was not significantly associated with vitamin B12 deficiency in the present analysis. This agrees with several earlier studies in which anthropometric measures showed weaker or inconsistent associations compared with duration and dose of metformin therapy [13,17,22]. The overall pattern suggests that treatment-related exposure is likely more important than body habitus in the development of

deficiency.

The biological explanation for these findings is well described in the literature. Metformin is thought to impair vitamin B12 absorption through interference with calcium-dependent uptake of the vitamin B12–intrinsic factor complex in the terminal ileum, and altered intestinal motility may also contribute [13,16,22]. The clinical relevance of this deficiency is considerable because vitamin B12 depletion may contribute to anemia, neuropathy, cognitive symptoms, and worsening of pre-existing diabetic neuropathy [15,16]. In the present study, the analysis focused on biochemical vitamin B12 deficiency rather than confirmed vitamin B12 deficiency anemia, because hematological indices were not included in the dataset. This distinction is important when interpreting the findings and framing the final title, objectives, and conclusions of the manuscript.

Taken together, the present findings support the growing evidence that metformin-treated patients with type 2 diabetes mellitus constitute a high-risk group for vitamin B12 deficiency, especially when treatment is prolonged. Current clinical sources also advise periodic vitamin B12 assessment in patients receiving long-term metformin, particularly when anemia or neuropathic symptoms are present [22]. In this context, the observed frequency of 31.3% in the present study provides a reasonable local basis for recommending greater vigilance, earlier screening, and timely supplementation where indicated. Further multicenter work with larger samples and inclusion of hematological and neurological outcomes would provide a more complete picture of the burden and consequences of this problem.

CONCLUSION

Vitamin B12 deficiency was a frequent finding among patients with type 2 diabetes mellitus receiving metformin therapy in this study. The deficiency was more common in older patients, females, and those with longer duration of diabetes and prolonged metformin use, while body mass index did not show a significant relationship. These findings indicate that metformin-associated vitamin B12 deficiency represents an important clinical problem that may remain unrecognized unless actively screened. Periodic monitoring of serum vitamin B12 should be considered, particularly in patients receiving long-term metformin, so that deficiency may be identified early and corrected before hematological or neurological complications develop.

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