



Metformin Therapy and Its Association with Hemoglobin and Vitamin B12 in Type 2 Diabetic Patients

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Abstract:

Background: Long-term metformin therapy in individuals with type 2 diabetes may influence hematological parameters and vitamin B₁₂ status. This study examines demographic, clinical, hematological, and family-history characteristics in metformin users versus non-users. **Methods:** Two groups were compared: 38 non-metformin users and 114 metformin users. Variables assessed included gender, age distribution, residence, metformin dosage/duration, side effects, dietary practices, hemoglobin, vitamin B₁₂ levels, and family history of diabetes, heart disease, and B₁₂ deficiency. **Results:** Both groups had equal gender distribution (50% male/female). Metformin users were older (mean age 56.7 ± 11.4 years vs. 46.2 ± 15.4 years in non-users). A majority of metformin users lived in rural areas (57%), compared to non-users (urban 28.9%). Among metformin users, 58.8% had been on treatment for over 10 years (mean duration 13.3 ± 8.4 years), with a mean daily dose of 720 mg. Most (88.6%) experienced no side effects; gastrointestinal symptoms were reported infrequently. Dietary avoidance of starchy/sugary foods was observed in 25.4%. Hemoglobin was normal in virtually all participants; 7.9% of metformin users exhibited low hemoglobin. Vitamin B₁₂ levels remained largely unknown (97% of metformin users and 100% of non-users), with only 0.9% showing normal B₁₂ and 1.8% low B₁₂ among users. Family history of type 2 diabetes and heart disease was slightly lower in metformin users compared to non-users. **Conclusion:** Metformin usability typically aligns with older age and rural residence. While hematological indices appear unchanged, unmeasured vitamin B₁₂ levels in the majority signal the need for routine assessment among metformin-treated populations, especially considering external evidence of deficiency risk with prolonged, high-dose use.

Keywords: Metformin, Type 2 Diabetes, Vitamin B₁₂, Hemoglobin.

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Introduction

Metformin remains the first-line pharmacological agent for managing type 2 diabetes mellitus (T2DM), endorsed by leading medical associations worldwide due to its proven efficacy, favorable safety profile, and cost-effectiveness [1]. However, accumulating evidence highlights a concerning link between prolonged metformin therapy and vitamin B₁₂ deficiency [2]. This adverse effect appears dose- and duration-dependent, with higher daily doses and extended treatment periods notably increasing the

risk [3]. The proposed mechanism involves impaired absorption of the vitamin B₁₂-intrinsic factor complex in the terminal ileum mediated by metformin's antagonistic interference with calcium-dependent uptake[4]. The clinical significance of reduced vitamin B₁₂ levels is substantial, given the vitamin's essential role in hematopoiesis and neurological function [5]. Deficiency may lead to megaloblastic anemia, peripheral neuropathy, cognitive decline, and other neuropsychiatric manifestations symptoms that often overlap with common diabetic complications, complicating accurate diagnosis [6]. Vitamin B12 is an important nutrient for



health. It can play a significant role in the formation of red blood cells and the functioning of the nervous system and brain. A deficiency of vitamin B₁₂ may increase the severity of diabetic neuropathy [7]. Deficiency of vitamin B₁₂ results in methylmalonyl-CoA accumulation that is subsequently converted to methylmalonic acid (MMA), whose levels in plasma are often high in individuals with a deficiency of vitamin B₁₂. In individuals with a deficiency of vitamin B₁₂, enhanced levels of homocysteine and MMA have been suggested to myelopathy leading to autonomic and peripheral neuropathy [8]. About 50% of individuals with T2DM will develop a certain degree of diabetic neuropathy throughout their life [9]. This group of patients has impaired clinical suspicion of vitamin B₁₂ deficiency based on neurological symptoms, which may result in an underdiagnosis of this serious vitamin deficit. If this condition is not properly diagnosed and treated, this can result in long-term neurological impairment [10]. Because large numbers of individuals with T2DM use metformin, the execution of a universal screening routine can enhance costs for the healthcare system [11]. Despite these insights, studies comparing metformin users and non-users in diverse populations remain limited. Examining both hematological parameters and vitamin B₁₂ status in tandem alongside demographic, clinical, and family-history factors can robustly inform clinical strategies for managing TDM patients on metformin.

Materials and Methods

This cross-sectional comparative study was conducted on 152 patients diagnosed with type 2 diabetes mellitus (T2DM) who were recruited from outpatient clinics in El-Beida, Libya. Patients were divided into two groups: Metformin group (n = 114): patients receiving metformin therapy for at least one year. Non-metformin group (n = 38): patients with T2DM managed without metformin. Inclusion criteria were: confirmed diagnosis of T2DM according to the American Diabetes Association (ADA) guidelines, age ≥ 20 years, and consent to participate in the study. Exclusion criteria included: type 1 diabetes, pregnancy, chronic kidney disease stage ≥ 3, liver disease, hematological disorders, or the use of vitamin B₁₂ supplements within the past 6 months. Data were collected using structured questionnaires and medical records. The following parameters were assessed: Demographic characteristics: age, sex, and place of residence (urban or rural). Clinical history: duration of diabetes, metformin use (dose and duration), side effects, dietary practices, and concurrent medications. Family history: presence of type 2 diabetes, cardiovascular disease, and vitamin B₁₂ deficiency. Venous blood samples were obtained from each participant after an overnight fast. Laboratory analyses included: Hemoglobin concentration (Hb): measured using an automated hematology analyzer (Sysmex XN-Series, Sysmex Corporation, Japan). Serum vitamin B₁₂ levels: determined using chemiluminescent immunoassay (Architect i2000SR, Abbott Diagnostics, USA).

Vitamin B₁₂ deficiency was defined as serum levels < 200 pg/mL, borderline levels 200–300 pg/mL, and normal > 300 pg/mL. Statistical Analysis Data were entered and analyzed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. Comparisons between groups were performed using the independent t-test for continuous variables and the Chi-square test (χ^2) for categorical variables. A p-value < 0.05 was considered statistically significant.

Results

The demographic and clinical characteristics presented in Table 1 highlight significant differences between metformin users and non-users that are essential for interpreting subsequent biochemical findings. Firstly, the gender distribution was well balanced across both groups (50% male and 50% female), which minimizes gender-related bias in the study population. However, the age distribution showed a distinct pattern: the non-metformin group consisted of relatively younger participants (mean age 46.21 ± 15.36 years), whereas the metformin group was significantly older (mean age 56.74 ± 11.36 years). This difference is consistent with the clinical practice that younger patients with early-stage T2DM may initially be managed with lifestyle modifications or alternative oral agents, while older patients with longer disease duration are more likely to require metformin therapy. Regarding place of residence, the majority of metformin users were from rural areas (57.0%), while non-users were predominantly from urban settings (28.9%). This may reflect variations in healthcare accessibility, prescribing practices, and lifestyle factors, which can influence both treatment choice and disease progression. The duration of metformin use was notably high, with 58.8% of patients reporting therapy for more than 10 years (mean duration 13.33 ± 8.36 years). Such prolonged exposure is clinically relevant, as it increases the risk of vitamin B₁₂ deficiency and hematological changes, which are central to the objectives of this study. The distribution of prescribed doses further demonstrates clinical variability, with most patients on the standard regimen of 500 mg twice daily (46.5%), while others received higher doses depending on glycemic control. Interestingly, the majority of metformin users (88.6%) reported no side effects upon initiation of therapy, confirming the generally favorable tolerability of the drug. Only a small fraction experienced mild gastrointestinal disturbances such as dizziness, vomiting, or diarrhea, which aligns with previously documented adverse effect profiles. Finally, dietary habits revealed that nearly three-quarters of metformin users (74.6%) did not adhere to a specific diet, while only 25.4% reported avoiding starchy and sugary foods. This lack of dietary regulation among a large proportion of patients could potentially exacerbate metabolic imbalance, despite pharmacological treatment, and may influence the long-term outcomes of diabetes management.

Table 1. General Characteristics of the Studied Population

		Non metformin group n(%)	Metformin group n(%)
Gender (n)	Male	19 (50.0)	57 (50.0)
	Female	19 (50.0)	57 (50.0)
Age	20- 34 years	8 (21.1)	5 (4.4)
	35 -49 years	15 (39.5)	19 (16.7)
	50 - 64 years	9 (23.7)	61 (53.5)
	65 - 80 years	6 (15.8)	29 (25.4)
	Mean $\pm$ SD*	46.21 $\pm$ 15.36	56.74 $\pm$ 11.36
Place of Residence:	Village	27 (21.1)	65 (57.0)
	City	11 (28.9)	49 (43.0)
Duration of Metformin use (years)	1- 5 years	-	24 (21.1)
	6 - 10 years	-	23 (20.2)
	More than 10 years	-	67 (58.8)
	Mean $\pm$ SD*	-	13.33 $\pm$ 8.36
Medication with metformin	Yes	-	114(0.75)
	No	38(0.25)	-
Metformin dose	500 mg/2 times daily	-	53 (46.5)
	850 mg/2 times daily	-	36 (34.6)
	1000 mg/1 times daily	-	25 (21.9)
	Mean $\pm$ SD*	-	720.18 $\pm$ 213.14
Side effects when you started taking metformin	Dizziness	-	5 (4.4)
	Vomiting	-	3 (2.6)
	Diarrhea	-	5 (4.4)
	No symptoms present	-	101 (88.6)
Dietary Practice	Does not follow any specific diet	-	85 (74.6)
	Avoids starchy and sugary foods	-	29 (25.4)

* These variables were categorized based on quartiles, (Mean $\pm$ SD)Standard deviation)

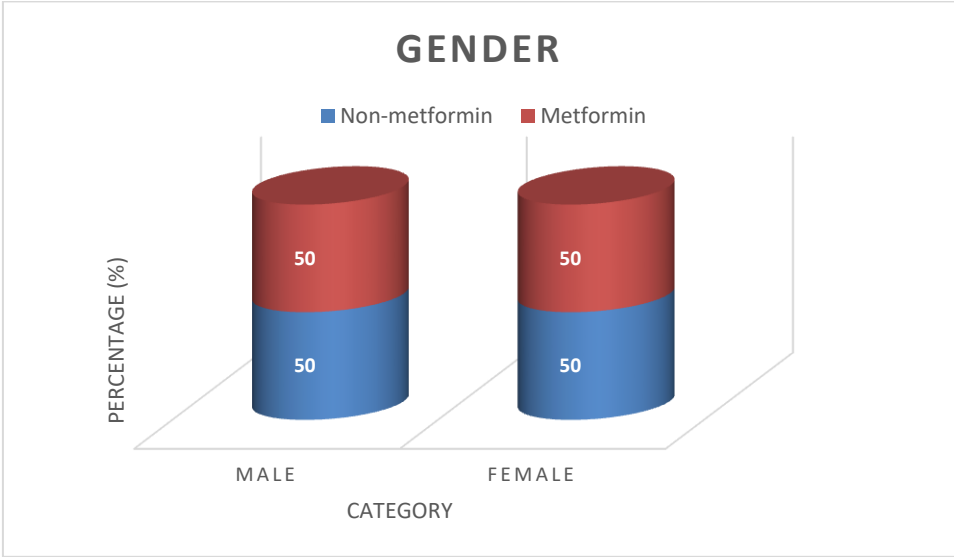


Figure 1. Comparison of Gender Proportions in Metformin and Non-Metformin Groups

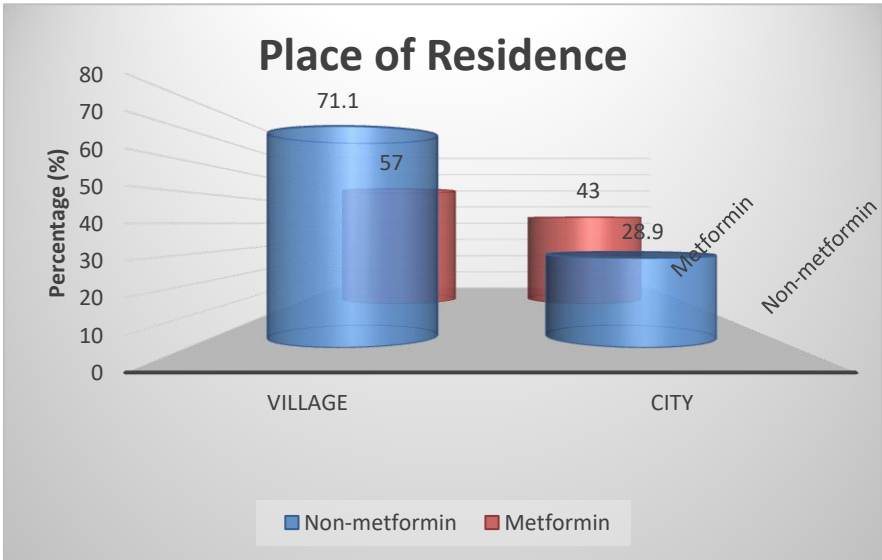


Figure 2. Comparison of Place of Residence between Metformin and Non-Metformin Groups”

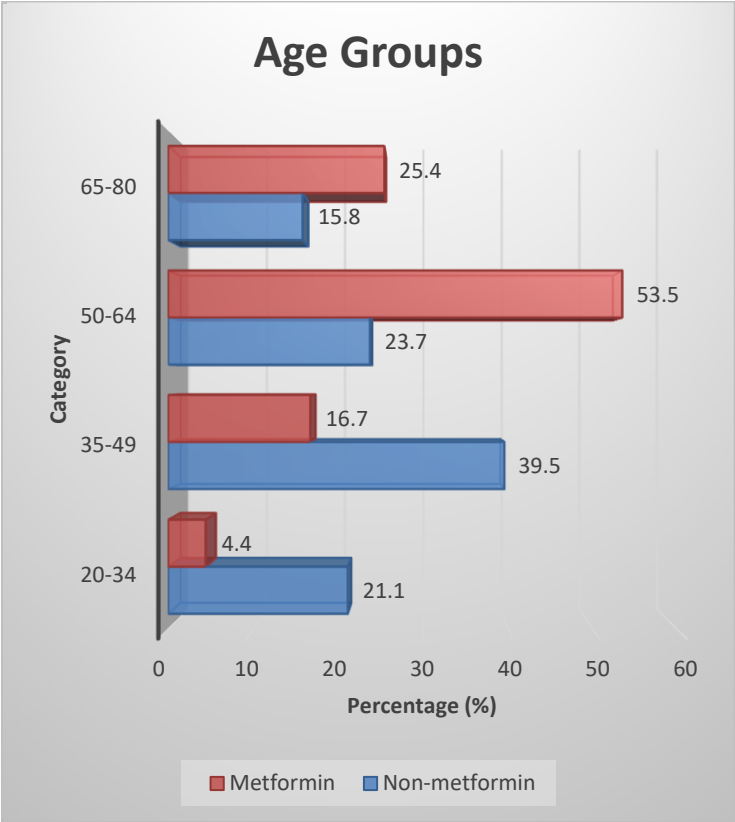


Figure 3. Comparison of Age Group Distribution between Metformin and Non-MetforminGroups

The findings presented in Table 2 provide valuable insights into the hematological and familial characteristics of the studied population, with particular emphasis on hemoglobin and vitamin B12 status in relation to metformin use. All participants in the non-metformin group demonstrated normal hemoglobin levels (100%), whereas 7.9% of the metformin group exhibited low hemoglobin concentrations. Although the majority of metformin users maintained normal values, the presence of anemia among this group is clinically significant, as it suggests a potential hematological impact of long-term metformin therapy. This observation aligns with earlier reports indicating that metformin may contribute to alterations in erythropoiesis through mechanisms linked to vitamin B12 deficiency and impaired folate metabolism. A striking observation is that none of the non-metformin patients had recorded vitamin B12 measurements, while in the metformin group, 97.4% were also undocumented (“unknown”), leaving only a very small fraction tested. Among those tested, 1.8% were found to have low B12 levels. Although the recorded prevalence is low, the lack of systematic screening severely underestimates the true frequency of B12 deficiency. This highlights a major gap in clinical practice, as international guidelines recommend periodic monitoring of vitamin B12 in patients on prolonged metformin therapy due to its well-documented association with malabsorption

of this vitamin. Regarding familial predispositions, family history of vitamin B12 deficiency was almost absent in both groups, indicating that the observed reductions are more likely drug-related rather than genetic. Family history of type 2 diabetes was highly prevalent across both groups, with the highest proportion reported from paternal inheritance (34.2% in non-users, 22.8% in users), followed by maternal or bilateral inheritance. This finding supports the strong genetic contribution to T2DM risk, which has been widely established in the literature. Family history of heart disease was reported in 26.3% of non-users and 12.3% of metformin users. Interestingly, the lower prevalence in the metformin group may suggest either a protective cardiovascular benefit associated with metformin therapy, consistent with its known cardiometabolic effects, or differences in familial risk background . The results of Table 2 demonstrate that while anemia and vitamin B12 deficiency appear relatively limited, their presence exclusively in the metformin group raises important clinical considerations. The very high rate of “unknown” B12 status indicates insufficient monitoring, which could obscure the actual impact of long-term metformin therapy on hematological health. These findings underscore the need for more systematic screening of vitamin B12 and hemoglobin levels in diabetic patients under chronic metformin therapy to prevent overlooked complications.

Table 2. General Characteristics of the Studied Population

	Non metformin group (38) *F (%)	Metformin group (114) F (%)
Last hemoglobin level reading		
Normal	38 (100)	105 (92.1)
Low	0 (0)	9 (7.9)
Unknown	0 (0)	0 (0)
Last Vitamin B12 level reading		
Normal	0 (0)	1 (0.9)
Low	0 (0)	2 (1.8)
Unknown	38 (100)	111 (97.4)
Family history of Vitamin B12 deficiency		
Yes	0 (0)	1 (0.9)
No	38 (100)	113 (99.1)
Family history of type 2 diabetes		
Yes, from the father's side	13 (34.2)	26 (22.8)
Yes, from the mother's side	7 (18.4)	22 (19.3)
Yes, from both sides	5 (13.2)	23 (20.2)
No	13 (34.2)	43 (37.3)
Family history of heart disease		
Yes	10 (26.3)	14 (12.3)
No	28 (73.7)	100 (87.7)

*F = Frequencies or observed counts

Discussion

The general characteristics of the studied population provide important context for interpreting the hematological and metabolic effects of metformin therapy in type 2 diabetic patients. The present study revealed equal proportions of males and females in both metformin and non-metformin groups. This is consistent with recent epidemiological reports that type 2 diabetes prevalence is nearly balanced between sexes in many populations, although some studies report a slightly higher risk among males due to differences in visceral adiposity and insulin sensitivity [12]. Therefore, the gender balance observed in our cohort reduces the likelihood of sex-related bias influencing metabolic outcomes. A key finding is the higher mean age of metformin users compared to non-users. Most metformin users were between 50–64 years, whereas younger age groups were more represented in the non-metformin group. This aligns with evidence that metformin is typically prescribed as first-line therapy in middle-aged and older adults with T2DM [13]. Age-related differences are clinically significant, as older patients are at greater risk of vitamin B12 deficiency and anemia, both of which are potential side effects of chronic metformin therapy [14]. Our findings demonstrate that a greater proportion of metformin users resided in villages (57%) compared to non-users (21.1%), while city residents were more

evenly distributed. Rural residence has been associated with reduced access to healthcare resources and dietary diversity, which may exacerbate the risk of nutrient deficiencies such as vitamin B12, particularly when combined with long-term metformin use[15]. This observation highlights the importance of considering socioeconomic and geographic factors when assessing treatment outcomes. A majority of metformin users (58.8%) had been on therapy for more than 10 years, with a mean duration of 13.3 years, reflecting the widespread use of metformin as a long-term therapy. Such prolonged exposure is clinically relevant, given that both dose and duration are strong predictors of vitamin B12 depletion and subsequent hematological changes [14]. The average daily dose was within therapeutic recommendations, and side effects were minimal, reported only in 11.4% of participants, consistent with the generally favorable gastrointestinal tolerability profile of metformin in long-term studies [16]. Nearly three-quarters of metformin users did not follow specific dietary restrictions, while 25.4% reported avoiding starchy and sugary foods. This dietary pattern may influence glycemic control and micronutrient status, potentially interacting with the metabolic effects of metformin. Previous studies suggest that dietary insufficiency of vitamin B12 and folate may potentiate metformin-induced deficiencies [17-19]. The results in Table 2 shed light on significant hematological and familial characteristics within type 2 diabetic patients,

differentiated by metformin usage. These findings align with existing literature and reinforce the importance of vigilant monitoring among long-term metformin users. While all non-metformin participants exhibited normal hemoglobin levels, 7.9 % of metformin users presented with anemia. This observation is consistent with evidence indicating an early risk of anemia associated with metformin therapy. Randomized controlled trials (ADOPT and UKPDS) have demonstrated initial hemoglobin declines in patients treated with metformin, and real-world data suggest that each 1 g/day increase in metformin dose correlates with a 2 % rise in annual anemia risk [20]. Although the exact mechanism remains undefined and likely multifactorial not solely attributable to vitamin B₁₂ deficiency this supports our finding of reduced hemoglobin among a subset of users. A striking outcome is that 97.4 % of metformin users and 100 % of non-users lacked recent vitamin B₁₂ testing (“unknown” status). Among those assessed, a small proportion (1.8 %) exhibited deficiency. This highlights a significant gap in surveillance practices. Global data indicate widespread B₁₂ deficiency among metformin-treated patients, with prevalence rates ranging from approximately 13 % to nearly 19 %. A recent Vietnamese study reported an 18.6 % prevalence of B₁₂ deficiency, strongly associated with both higher metformin dose and longer usage duration [21]. Additionally, evidence shows that therapy exceeding four years leads to a 41 % higher odds of B₁₂ deficiency. Given that the majority of our metformin users have prolonged exposure, the lack of routine testing suggests a high probability of undetected deficiency [14]. Family history of vitamin B₁₂ deficiency was nearly absent, indicating that observed hematological changes are more likely attributable to pharmacologic effects rather than genetic predisposition. Both groups reported considerable rates of familial T2DM, reinforcing the hereditary contribution to disease development. Interestingly, family history of heart disease was higher in non-users (26.3 %) compared to metformin users (12.3 %). While speculative, this aligns with metformin’s reported cardioprotective properties and potential amelioration of familial cardiovascular risk, meriting further exploration [22]. Collectively, these findings underscore a critical oversight in routine vitamin B₁₂ and hemoglobin monitoring among metformin-treated diabetic patients. Regulatory health authorities now classify B₁₂ deficiency as a common adverse effect of metformin particularly in high-dose or long-term users—and advise regular serum level surveillance. The data imply that a sizeable portion of patients may harbor latent anemia or B₁₂ deficiency, risking neurological and hematological complications, especially within populations lacking systematic follow-up[23].

Conclusion

This study highlights that long-term metformin therapy among type 2 diabetic patients is more common in older individuals and those residing in rural areas. While hemoglobin levels were largely preserved, a small proportion of metformin users exhibited anemia, indicating a possible hematological effect of chronic therapy. Importantly, the lack of routine vitamin B₁₂ testing in the majority of patients underscores a critical gap in clinical monitoring, which may mask the true prevalence of deficiency. Given the established association between prolonged metformin use and vitamin B₁₂ depletion, periodic screening for vitamin B₁₂ and hemoglobin is strongly recommended to prevent unrecognized anemia and neurological complications. These findings reinforce the necessity

of integrating nutritional surveillance into diabetes management protocols, particularly for patients on high-dose or long-term metformin therapy.

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