Patient-Reported Vitamin B12 Deficiency, Peripheral Neuropathy and Monitoring Gaps Among People with Diabetes in Punjab, Pakistan: A Cross-Sectional Survey

Hafiz Muhammad Amar[1*], Muhammad Hamid Khan[1*], Shayan Ali[1], Abdus Saboor[1], Muneeb Ahmad[1], Aousaf Ahmad[1], Sitara Kiran[2], Muhammad Adeel Arshad[3], Shoaib Nawaz[4], Sidra Huma[5], Shakeel Ijaz[6*]

[1]Quaid-e-Azam College of Pharmacy, Sahiwal 57000, Punjab, Pakistan
[2]Health and Population Department, Govt. of Punjab, Lahore, Pakistan
[3]Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical and Allied Health Science, Lahore College for Women University, Lahore, Pakistan
[4]Department of Pharmacy, The University of Lahore, Sargodha campus, Sargodha 40100, Pakistan
[5]Bahawalpur College of Pharmacy, Bahawalpur Medical and Dental College, Bahawalpur 63100, Pakistan
[6]Central Park College of Pharmacy, Lahore 54600, Pakistan

ARTICLE INFO

***Correspondence to**
Shakeel Ijaz
Central Park College of Pharmacy, Lahore, Pakistan
Email: shakeelijaz80@gmail.com
Hafiz Muhammad Amar
Quaid-e-Azam College of Pharmacy, Sahiwal, Pakistan
Email: amerhabib.ah@gmail.com
Muhammad Hamid Khan
Quaid-e-Azam College of Pharmacy, Sahiwal, Pakistan
Email: ahmy8020@gmail.com

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ABSTRACT

Background: Long-term metformin therapy is associated with vitamin B12 deficiency and potential neurological complications. This study assessed self-reported vitamin B12 deficiency, peripheral neuropathy, and awareness of vitamin B12 status among adults in Punjab, Pakistan.

Methods: This cross-sectional study included 205 adults recruited from hospitals, clinics, and pharmacies across four districts of Punjab. A structured questionnaire collected demographic information, diabetes status, metformin exposure, previous vitamin B12 deficiency diagnoses, and peripheral neuropathy history. Clinical outcomes were self-reported without biochemical confirmation. Associations were evaluated using chi-square and Fisher's exact tests, with unadjusted odds ratios (ORs) and 95% confidence intervals (CIs).

Results: Among 205 participants, 164 (80.0%) reported metformin use, and 164 (80.0%) had diabetes. Vitamin B12 deficiency was reported by 148 (72.2%), while 35 (17.1%) were unaware of their status. Among participants with known status, deficiency was higher among metformin users than non-users (93.2% vs. 45.5%; OR = 16.56, 95% CI: 5.76–47.63; $p < 0.001$). Uncertainty was greater among non-users (46.3% vs. 9.8%; OR = 7.99, 95% CI: 3.58–17.81; $p < 0.001$). Reported neuropathy was higher among participants with diabetes (85.8% vs. 27.6%; $p < 0.001$), without significant differences between diabetes types ($p = 0.128$).

Conclusion: Metformin use was associated with reported vitamin B12 deficiency and greater diagnostic awareness. Differential screening may influence these associations. Biochemical confirmation and standardized neuropathy assessment are necessary.

Keywords: Vitamin B12 deficiency; Metformin; Diabetes mellitus; Peripheral neuropathy; Screening awareness; Pakistan.

INTRODUCTION

Metformin is widely prescribed as a first-line pharmacological treatment for type 2 diabetes mellitus because of its established glucose-lowering efficacy, favorable safety profile, and affordability. Despite its therapeutic benefits, prolonged metformin exposure has been consistently associated with reduced serum vitamin B12 concentrations, raising concerns regarding its potential contribution to neurological complications. The relationship between metformin treatment and impaired vitamin B12 absorption was first described by Berchtold and colleagues in 1969, providing early evidence of an adverse effect on intestinal nutrient absorption. [1] Subsequent investigations have strengthened this association through randomized controlled trials and long-term observational studies. In a placebo-controlled trial involving 390 patients followed for approximately 4.3 years, metformin treatment was associated with a 19% reduction in serum vitamin B12 concentrations and a 7.2-percentage-point increase in the absolute risk of biochemical deficiency compared with placebo.[2]

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Similarly, the Diabetes Prevention Program Outcomes Study reported vitamin B12 deficiency in 4.3% of metformin-treated participants after five years and 7.4% after thirteen years, compared with 2.3% and 5.4%, respectively, among placebo recipients.[3] These findings support periodic vitamin B12 assessment during prolonged metformin therapy, particularly among patients presenting with neurological symptoms or additional risk factors for deficiency.

Although the association between metformin exposure and biochemical vitamin B12 deficiency is well documented, its clinical implications remain incompletely understood. Vitamin B12 deficiency may produce neurological manifestations that overlap with diabetic peripheral neuropathy, including sensory disturbances, paresthesia, numbness, and impaired peripheral nerve function. This clinical similarity creates a diagnostic challenge because neurological symptoms resulting from a potentially treatable nutritional deficiency may be attributed exclusively to diabetes-related nerve damage.[4] Wile and Toth demonstrated that metformin exposure was associated with elevated homocysteine and methylmalonic acid concentrations and greater severity of peripheral neuropathy, with findings suggesting a relationship between cumulative metformin exposure and neurological impairment.[5] Nevertheless, subsequent investigations have produced inconsistent results. A South African study found peripheral neuropathy in 36.8% of vitamin B12-replete participants and 32.3% of vitamin B12-deficient participants, without demonstrating a significant association between serum vitamin B12 concentrations and neuropathy symptoms. [6] Likewise, research conducted in Saudi Arabia identified a substantially increased likelihood of vitamin B12 deficiency among metformin users but did not establish a corresponding increase in peripheral neuropathy. [7]

Additional investigations from Egypt and Botswana have similarly reported limited or inconsistent associations between vitamin B12 deficiency and diabetic peripheral neuropathy, suggesting that neurological complications may be influenced by multiple clinical factors beyond vitamin B12 status alone.[8,9] In contrast, a multicenter investigation conducted in Karachi, Pakistan, identified differences in neuropathy-related clinical measures among patients receiving metformin and those with vitamin B12 deficiency, highlighting the importance of further investigation in local populations.[10] Recent studies have continued to examine the relationship between vitamin B12 depletion, metformin exposure, and diabetic neurological complications, indicating that the underlying association remains clinically relevant but requires careful consideration of potential confounding factors.[11,12]

The reported prevalence of vitamin B12 deficiency among patients with diabetes also varies considerably across geographical settings and study populations. Biochemically assessed prevalence estimates include approximately 6.6% in Botswana, 7.8% in Saudi Arabia, 28.1% in South Africa, and 28.3% in Malaysia. [6,7,9,13] Such variation may reflect differences in laboratory methods, diagnostic thresholds, dietary patterns, patient characteristics, metformin dosage, and treatment duration. Research evaluating metformin-related vitamin B12 deficiency has also demonstrated the importance of medication dosage and duration when interpreting deficiency risk. [11,14] However, most previous investigations have concentrated on laboratory-confirmed deficiency among participants who underwent biochemical testing. Comparatively less attention has been directed toward patients' awareness of their vitamin B12 status, previous testing experiences, and potential differences in diagnostic ascertainment between metformin users and non-users.

Pakistan faces a substantial burden of diabetes mellitus, emphasizing the importance of identifying potentially preventable complications and gaps in routine clinical assessment. The Second National Diabetes Survey of Pakistan documented a considerable prevalence of diabetes and associated metabolic risk factors, highlighting the growing need for accessible and effective diabetes-related healthcare services.[15] Although previous Pakistani investigations have examined metformin-associated vitamin B12 deficiency, evidence regarding patient awareness of vitamin B12 status and differences in reported diagnosis according to metformin exposure remains comparatively limited.[10,16] Moreover, studies relying exclusively on biochemical assessment do not directly describe the experiences of individuals who have never undergone vitamin B12 testing or who remain unaware of their diagnostic status.

To address this gap, the present cross-sectional survey was conducted among individuals with and without diabetes across four districts of Punjab, Pakistan. The study examined self-reported histories of vitamin B12 deficiency, peripheral neuropathy, and metformin use, with particular emphasis on differences in awareness of vitamin B12 status between treatment groups. The primary objective was to characterize the reported burden of these conditions and evaluate their associations with metformin exposure and diabetes status. Importantly, the study was designed to assess self-reported diagnostic history rather than establish laboratory-confirmed vitamin B12 deficiency or determine causal relationships. By examining both reported diagnoses and uncertainty regarding vitamin B12 status, the findings may provide insight into potential screening disparities and identify areas requiring improved clinical assessment and further prospective investigation.

METHODOLOGY

A cross-sectional, questionnaire-based study was conducted between May and October 2024 among adults attending selected hospitals, healthcare clinics, and community pharmacies in Sahiwal, Pakpattan, Chichawatni, and Okara, Punjab, Pakistan. The study aimed to investigate self-reported vitamin B12 deficiency, peripheral neuropathy, and awareness of vitamin B12 status in relation to metformin use and diabetes status. Participants were recruited through consecutive, non-probability sampling at the participating healthcare facilities. A target sample size of 205 participants was established using a single-population proportion formula based on an assumed prevalence. Adults attending the selected sites were approached and invited to participate voluntarily, regardless of their diabetes status. Consequently, the study population included individuals reporting type 1 diabetes mellitus, type 2 diabetes mellitus, prediabetes, no diabetes, or uncertain diabetes status. Participants without diabetes served as an internal comparison group for evaluating differences in reported neurological complications.

Data were collected using a purpose-designed, interviewer-administered structured questionnaire developed in accessible language to facilitate comprehension among participants with varying educational backgrounds. The questionnaire recorded sociodemographic characteristics, including sex, age category, weight category, marital status, occupation, monthly household income, district, residential area, and educational attainment. Clinical information included self-reported diabetes status and classification, current metformin use, previous diagnosis of vitamin B12 deficiency, frequency of symptoms attributed to vitamin B12 deficiency, history of peripheral neuropathy, and medication use for neuropathic pain. Vitamin B12 deficiency was assessed through participants' responses regarding previous clinical diagnosis, categorized as currently diagnosed, previously

diagnosed, never diagnosed, or uncertain. Participants reporting either current or previous vitamin B12 deficiency were classified as having a history of diagnosed deficiency. Peripheral neuropathy was assessed through a separate question concerning previous diagnosis by a healthcare professional, with responses categorized as confirmed, absent, suspected but unconfirmed, or unknown. Participants reporting uncertain diagnostic status were retained as a separate category rather than automatically classified as negative.

All clinical variables were based exclusively on participant-reported information. No venous blood samples were obtained, and biochemical parameters, including serum vitamin B12, homocysteine, methylmalonic acid, hemoglobin, and glycated hemoglobin, were not measured. Furthermore, peripheral neuropathy was not independently confirmed through neurological examination, nerve conduction studies, or validated assessment instruments. Information regarding metformin dosage and treatment duration was unavailable, preventing evaluation of potential dose-dependent or duration-related associations. Accordingly, the study outcomes represent reported diagnostic histories and awareness rather than laboratory-confirmed vitamin B12 deficiency or objectively established peripheral neuropathy.

Ethical approval was granted by the Research and Ethics Committee of Quaid-e-Azam College of Pharmacy, Sahiwal, Pakistan (Approval No. QACP/24/355). Before data collection, participants were verbally informed about the study's purpose and invited to participate voluntarily. The questionnaire was administered following the provision of study information, and responses were used for research analysis.

Data were entered and organized using IBM SPSS Statistics, version 25, while statistical analyses were performed using Python 3 with SciPy 1.18 and statsmodels 0.15. Categorical variables were summarized using frequencies and percentages, with corresponding Wilson 95% confidence intervals calculated for proportions. Associations between categorical variables were evaluated using Pearson's chi-square test, while Fisher's exact test was applied when expected cell frequencies were below five. For comparisons involving four-category outcomes across two exposure groups, an initial omnibus chi-square test was followed by category-specific Fisher's exact tests with Bonferroni correction for multiple comparisons. Effect sizes were expressed using Cramér's V for contingency tables and unadjusted odds ratios with Woolf 95% confidence intervals for binary comparisons. All statistical tests were two-sided, with p-values below 0.05 considered statistically significant. Since age and weight were recorded as categorical intervals, continuous summary measures such as means and standard deviations were not calculated. Multivariable regression was not performed because individual-level covariate data were unavailable in the analytical dataset; therefore, all reported odds ratios represent crude associations without adjustment for potential confounding factors.

RESULTS

Participant characteristics

Of the 205 participants, 105 (51.2%) were men and 100 (48.8%) were women. Age was concentrated in middle life, with 105 (51.2%) in the 49-58-year band and 52 (25.4%) in the 39-48-year band; 47 participants (22.9%) were 59 years or older and one (0.5%) was under 39. Weight was most often in the 79-91 kg band (92 participants, 44.9%) or the 63-78 kg band (75, 36.6%). Residence was predominantly urban (156, 76.1%). Full characteristics appear in Table 1.

Table 1. Demographic characteristics of the participants (n = 205).

Characteristic	Category	n	%	95% CI (%)
Sex	Male	105	51.2	44.4–58.0
	Female	100	48.8	42.0–55.6
Age (years)	29–38	1	0.5	0.1–2.7
	39–48	52	25.4	19.9–31.7
	49–58	105	51.2	44.4–58.0
	59–68	22	10.7	7.2–15.7
	69–78	25	12.2	8.4–17.4
Weight (kg)	37–50	6	2.9	1.3–6.2
	51–62	25	12.2	8.4–17.4
	63–78	75	36.6	30.3–43.4
	79–91	92	44.9	38.2–51.7
	92–102	7	3.4	1.7–6.9
Residence	Urban	156	76.1	69.8–81.4
	Rural	49	23.9	18.6–30.2

(Confidence intervals are Wilson intervals for a single proportion. Age and weight were recorded in bands, so no mean or standard deviation can be derived. Percentages may not sum to 100 because of rounding).

Reported diabetes, vitamin B12 and neuropathy status

Type 2 diabetes was reported by 136 participants (66.3%) and type 1 by 28 (13.7%); 2 reported pre-diabetes, 36 reported no diabetes and 3 were unsure (Table 2). Metformin was being taken by 164 participants (80.0%).

Table 2. Self-reported diabetes status, vitamin B12 deficiency status, symptom frequency and peripheral neuropathy status (n = 205).

Variable	Response	n	%
Diabetes status	Type 2 diabetes	136	66.3
	Type 1 diabetes	28	13.7
	Pre-diabetes	2	1.0
	No diabetes	36	17.6
	Unsure	3	1.5
Metformin use	Yes	164	80.0
	No	41	20.0
Ever diagnosed with B12 deficiency	Yes, currently	31	15.1
	Yes, in the past	117	57.1
	No, never	22	10.7
	Unsure	35	17.1
Frequency of B12-related symptoms	Frequently	29	14.1
	Occasionally	133	64.9
	Rarely	27	13.2
	Never	16	7.8
Peripheral neuropathy diagnosed	Yes	135	65.9
	No	43	21.0
	Suspected but not confirmed	17	8.3
	Do not know	10	4.9
Medication for nerve pain	Yes, regularly	119	58.0
	Yes, occasionally	30	14.6
	Not now, but previously	12	5.9
	Never	44	21.5

(Symptoms were those the participant attributed to vitamin B12 deficiency, such as fatigue, numbness and memory difficulty. All items are patient-reported; no variable in this table was confirmed biochemically or by record review)

A diagnosis of vitamin B12 deficiency had been received at some point by 148 participants (72.2%; 95% CI 65.7-77.9), comprising 31 (15.1%) currently affected and 117 (57.1%) affected in the

past. Twenty-two (10.7%) reported never having been diagnosed and 35 (17.1%) did not know. Symptoms attributed to B12 deficiency were reported occasionally by 133 (64.9%) and frequently by 29 (14.1%), with 16 (7.8%) reporting none.

Peripheral neuropathy had been diagnosed in 135 participants (65.9%), was suspected but unconfirmed in 17 (8.3%), and was absent in 43 (21.0%); 10 (4.9%) did not know. Medication for nerve pain was taken regularly by 119 participants (58.0%) and occasionally by 30 (14.6%).

Metformin use and reported vitamin B12 deficiency

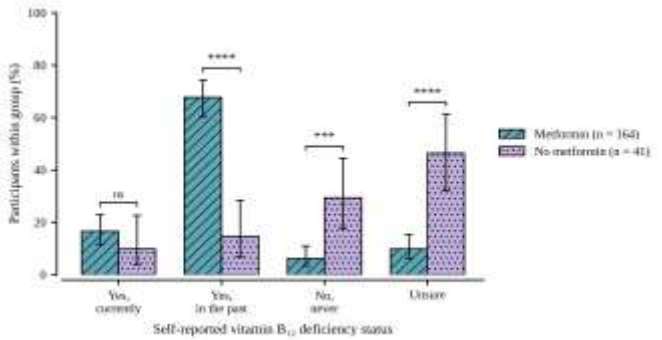
The distribution of reported B12 status differed markedly between metformin users and non-users (χ^2 = 59.27, df = 3, p < 0.001; Cramér's V = 0.54; Table 3, Figure 1). The difference was driven by two categories. A past diagnosis was reported by 111 of 164 metformin users (67.7%, 95% CI 60.2-74.4) against 6 of 41 non-users (14.6%, 95% CI 6.9–28.4; adjusted p < 0.001). Conversely, 19 of 41 non-users (46.3%, 95% CI 32.1-61.3) did not know their status, compared with 16 of 164 users (9.8%, 95% CI 6.1–15.3; adjusted p < 0.001). Current deficiency did not differ significantly between the groups (16.5% versus 9.8%; adjusted p > 0.99).

Table 3. Self-reported vitamin B12 deficiency status by metformin use.

B12 deficiency status	Metformin, n=164 (%)	No metformin, n=41 (%)	Fisher exact p	Adjusted p
Yes, currently	27 (16.5)	4 (9.8)	0.339	> 0.99
Yes, in the past	111 (67.7)	6 (14.6)	< 0.001	< 0.001
No, never	10 (6.1)	12 (29.3)	< 0.001	< 0.001
Unsure	16 (9.8)	19 (46.3)	< 0.001	< 0.001

(Omnibus test across the four categories: χ^2 = 59.27, df = 3, p < 0.001; Cramér's V = 0.54. Adjusted p values are Bonferroni-corrected for four comparisons. Restricting to participants who gave a definite answer, 138 of 148 metformin users (93.2%) and 10 of 22 non-users (45.5%) reported ever having been diagnosed (unadjusted OR 16.56, 95% CI 5.76–47.63). The odds of being unsure of B12 status were 7.99 times higher among non-users (95% CI 3.58–17.81). All odds ratios are unadjusted).

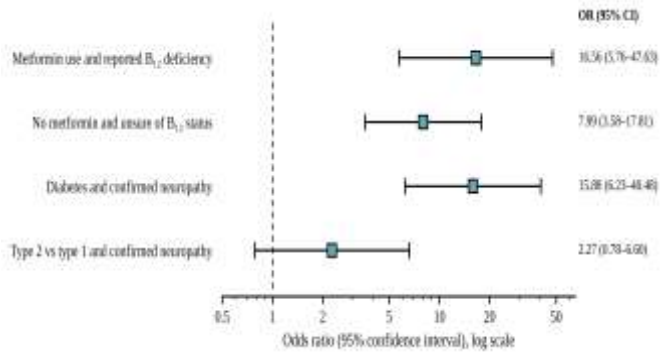
Figure 1. Self-reported vitamin B12 deficiency status among metformin users (n = 164) and non-users (n = 41).



(Bars show the percentage of participants within each exposure group; error bars are Wilson 95% confidence intervals for a single proportion. Categories were compared using the Fisher exact test with Bonferroni adjustment for four comparisons: ns, not significant; ***p < 0.001; ****p < 0.0001. The omnibus comparison across all four categories gave χ^2 = 59.27, df = 3, p < 0.001, Cramér's V = 0.54. All statuses are patient-reported; serum vitamin B12 was not measured)

Restricting the analysis to participants who gave a definite answer, 138 of 148 metformin users (93.2%) and 10 of 22 non-users (45.5%) reported ever having been diagnosed, giving an unadjusted odds ratio of 16.56 (95% CI 5.76-47.63; p < 0.001). The odds of being unsure of one's B12 status were 7.99 times higher among those not taking metformin (95% CI 3.58-17.81; p < 0.001). Both estimates are shown with the neuropathy contrasts in Figure 2.

Figure 2. Unadjusted odds ratios with 95% confidence intervals for the four principal contrasts.



(Squares mark the point estimate and horizontal lines the confidence interval; the dashed vertical line marks an odds ratio of the horizontal axis is logarithmic. Confidence intervals were calculated by the Woolf method. No estimate is adjusted for age, diabetes duration or any other covariate, because individual-level data were not available for modelling).

Diabetes status and peripheral neuropathy

Among participants giving a definite answer, confirmed peripheral neuropathy was reported by 127 of 148 with type 1 or type 2 diabetes (85.8%) and by 8 of 29 without diabetes (27.6%; Fisher p < 0.001; OR 15.88, 95% CI 6.23-40.48). By type, 108 of 136 participants with type 2 diabetes (79.4%, 95% CI 71.9-85.4) and 19 of 28 with type 1 (67.9%, 95% CI 49.3-82.1) had a confirmed diagnosis, compared with 8 of 36 participants without diabetes (22.2%, 95% CI 11.7–38.1). The difference between type 2 and type 1 was not statistically significant (Fisher p = 0.128; OR 2.27, 95% CI 0.78-6.60), whereas both differed from the group without diabetes (p < 0.001 and p < 0.001 respectively; Table 4, Figure 3).

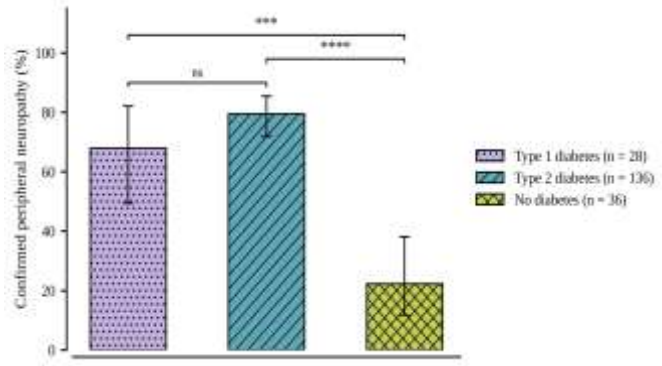
Table 4. Peripheral neuropathy status by self-reported diabetes status.

Diabetes status	Yes	No	Suspected	Do not know	Total	Confirmed, % (95% CI)
Type 1 diabetes	19	6	2	1	28	67.9 (49.3–82.1)
Type 2 diabetes	108	15	9	4	136	79.4 (71.9–85.4)

Pre-diabetes	0	0	1	1	2	0.0 (0.0–65.8)
No diabetes	8	21	3	4	36	22.2 (11.7–38.1)
Unsure	0	1	2	0	3	0.0 (0.0–56.1)
Total	135	43	17	10	205	—

(Among participants giving a definite answer, confirmed neuropathy was reported by 127 of 148 with type 1 or type 2 diabetes (85.8%) and 8 of 29 without diabetes (27.6%; Fisher p < 0.001; OR 15.88, 95% CI 6.23–40.48). Type 2 and type 1 did not differ significantly (Fisher p = 0.128; OR 2.27, 95% CI 0.78–6.60). The pre-diabetes and unsure rows are shown for completeness; with 2 and 3 participants they support no inference).

Figure 3. Confirmed peripheral neuropathy by self-reported diabetes status.



(Bars show the percentage within each group reporting a clinician-confirmed diagnosis; error bars are Wilson 95% confidence intervals. Groups were compared using the Fisher exact test: ns, not significant; ***p < 0.001; ****p < 0.0001. Participants reporting pre-diabetes (n = 2) and uncertain diabetes status (n = 3) are omitted from the figure and reported in Table 4. Neuropathy status is patient-reported; no validated neuropathy instrument was administered).

DISCUSSION

The present study identified two important and interrelated findings concerning metformin exposure, self-reported vitamin B12 deficiency, and peripheral neuropathy among patients with diabetes mellitus. First, participants receiving metformin were substantially more likely to report a previous diagnosis of vitamin B12 deficiency. Second, awareness of vitamin B12 status differed considerably between metformin users and non-users, raising concerns regarding differential screening practices, diagnostic ascertainment, and the interpretation of the observed associations.

The higher frequency of reported vitamin B12 deficiency among metformin users is consistent with previous clinical and epidemiological investigations demonstrating an association between prolonged metformin exposure and reduced vitamin B12 concentrations. Randomized controlled trials and longitudinal studies have established that sustained metformin therapy increases the likelihood of biochemical vitamin B12 deficiency. [2,3] Similar findings have been reported in cross-sectional investigations conducted in Saudi Arabia, Malaysia, and other populations with type 2 diabetes mellitus. [7,13] The present study demonstrated a particularly strong association, with an odds ratio of 16.56. However, this estimate substantially exceeds the odds ratio of approximately 4.72 reported in the Saudi population. [7] Such a discrepancy should not necessarily be interpreted as evidence of a stronger biological effect in the present population. Rather, differences in diagnostic methods, participant selection, and previous testing practices may have contributed to the observed magnitude of association. Recent reviews have similarly emphasized the importance of evaluating metformin-related vitamin B12 deficiency within the broader context of patient characteristics, medication exposure, and screening practices. [17] Additional clinical investigations have documented vitamin B12 deficiency among metformin-treated patients, while recent biochemical research continues to examine the relationship between metformin exposure and vitamin status. [18,19]

A particularly important finding was the considerable difference in participants' awareness of their vitamin B12 status according to metformin exposure. Approximately 46.3% of non-metformin users were unable to identify whether they had previously experienced vitamin B12 deficiency, compared with approximately 10% of metformin users. This discrepancy suggests possible differences in access to, or utilization of, vitamin B12 screening between treatment groups. Patients receiving metformin may be more frequently advised to undergo vitamin B12 assessment because of its recognized association with vitamin B12 depletion, whereas individuals managed with alternative glucose-lowering medications or lifestyle interventions may receive less attention regarding nutritional screening. Consequently, vitamin B12 deficiency may be more frequently identified among metformin users because they are more likely to undergo testing. This possibility introduces differential ascertainment bias, which may exaggerate the observed association between metformin exposure and previously diagnosed vitamin B12 deficiency. Recent reviews have emphasized the importance of systematic clinical assessment, appropriate diagnostic evaluation, and consideration of metformin exposure when identifying vitamin B12 deficiency. [20,21,22]

Importantly, vitamin B12 deficiency among patients with diabetes cannot be attributed exclusively to metformin therapy. Other contributing factors may include advancing age, inadequate dietary intake, gastrointestinal malabsorption, and prolonged exposure to gastric acid-suppressing medications. Previous research conducted in Botswana demonstrated that additional clinical and medication-related factors may contribute to vitamin B12 deficiency independently of metformin exposure. [9] Therefore, restricting vitamin B12 assessment primarily to metformin users may result in the underrecognition of deficiency among patients receiving alternative treatments. The substantial proportion of non-metformin users who were unaware of their vitamin B12 status highlights a potentially important gap in clinical assessment. Nevertheless, the present study cannot determine whether these individuals were biochemically deficient because laboratory measurements were not performed. The overall prevalence of self-reported vitamin B12 deficiency was 72.2%, considerably exceeding estimates reported in several previous studies using biochemical confirmation. Published investigations have documented substantially lower frequencies, although prevalence varies according to population characteristics, diagnostic thresholds, metformin exposure, and laboratory methods. [6,7,9,13] This discrepancy requires cautious interpretation. Unlike studies measuring serum vitamin B12 concentrations, the present investigation relied on participants' recollection of previous diagnoses. Consequently, the reported proportion reflects a history of diagnosed deficiency rather than current biochemical prevalence. Facility- and pharmacy-based recruitment may also have disproportionately included

individuals with established complications, greater healthcare utilization, or previous diagnostic investigations. Recall bias, previous supplementation, and differences in healthcare access may have further influenced responses. Evidence from Pakistani investigations and more recent large-scale observational research demonstrates the continued relevance of evaluating vitamin B12 status among metformin-treated populations, although differences in study design and measurement must be considered when comparing findings. [23,24]

Peripheral neuropathy was another frequently reported clinical condition, particularly among participants with type 2 diabetes mellitus. Approximately 79.4% of participants with type 2 diabetes reported a previous diagnosis of peripheral neuropathy. This proportion exceeds estimates frequently described in clinical investigations employing structured neuropathy assessments. [5,6,12] Several factors may explain this difference. Recruitment from healthcare facilities and pharmacies may have increased the representation of patients with established neurological symptoms or complications. Furthermore, approximately 72.7% of participants were receiving medication for neuropathic pain, indicating that individuals with existing neurological complaints were particularly prominent in the sample. The absence of standardized clinical examination also prevented independent confirmation of neuropathy and differentiation from other neurological disorders. Previous investigations have highlighted the importance of biochemical assessment and objective neurological evaluation when examining the potential contribution of vitamin B12 deficiency to diabetic neuropathy. [25,26] Therefore, the present findings should be interpreted as the frequency of reported neuropathy diagnoses within the recruited sample rather than the prevalence of confirmed diabetic peripheral neuropathy in the wider population.

Although neuropathy appeared more frequent among participants with type 2 diabetes, the difference between type 1 and type 2 diabetes did not achieve statistical significance ($p = 0.128$). This result should not be interpreted as evidence that diabetes type has no relationship with neuropathy risk. Only 28 participants had type 1 diabetes, resulting in an unequal distribution between comparison groups and potentially limiting statistical power. Furthermore, age, diabetes duration, glycemic control, medication exposure, and other metabolic factors may independently influence peripheral nerve dysfunction. Without appropriate adjustment for these potential confounders, the observed differences cannot be attributed exclusively to diabetes classification.

The possible contribution of vitamin B12 deficiency to peripheral neuropathy remains clinically important but could not be directly established in the present investigation. Earlier evidence indicated an association between metformin exposure, elevated homocysteine and methylmalonic acid concentrations, and worsening peripheral neuropathy.[5] However, subsequent studies have produced inconsistent findings regarding the strength and clinical significance of this relationship.[6,7,8,9,10] Recent literature has continued to emphasize the potential neurological consequences of metformin-associated vitamin B12 depletion, including its relevance to distal symmetrical and autonomic neuropathy.[27] Contemporary analyses have also identified vitamin B12 deficiency as a potentially underrecognized contributor to neurological manifestations among patients receiving long-term metformin treatment.[28] A recent systematic review and meta-analysis further reflects the ongoing interest in clarifying the relationship between metformin exposure, vitamin B12 deficiency, and peripheral neuropathy.[29] Additionally, randomized clinical evidence indicates that vitamin B12 supplementation may improve selected neuropathy-related outcomes in appropriately selected patients, although such benefits should not be generalized to all patients with diabetic neuropathy.[30]

From a clinical perspective, the present findings emphasize the importance of improving awareness of vitamin B12 assessment among patients with diabetes, particularly individuals receiving prolonged metformin treatment. However, the findings do not establish that metformin caused vitamin B12 deficiency or that deficiency contributed directly to peripheral neuropathy in this population. The pronounced difference in awareness of vitamin B12 status between treatment groups suggests that screening practices themselves may influence the observed strength of association. Future investigations should incorporate biochemical confirmation, standardized neuropathy assessment, detailed medication exposure, and adjustment for relevant confounding variables. Such approaches would provide more reliable evidence regarding the relationships among metformin use, vitamin B12 deficiency, and diabetic neurological complications.

Several limitations should be considered when interpreting the findings. Vitamin B12 deficiency and peripheral neuropathy were assessed through self-reported diagnoses without biochemical confirmation or validated neurological instruments, increasing the risk of recall bias and misclassification. Metformin dosage and treatment duration were not recorded, preventing assessment of dose–response relationships. [3,11,14] Furthermore, the cross-sectional design precluded causal interpretation. Recruitment from healthcare facilities and pharmacies may have introduced selection bias, limiting the generalizability of the findings. Unequal representation of diabetes types and the absence of information on dietary intake, supplementation, glycemic control, and other potential confounders further restricted the analysis. Differences in vitamin B12 testing and diagnostic awareness between metformin users and non-users may also have influenced the observed associations. Future prospective studies incorporating laboratory confirmation, standardized neuropathy assessments, and multivariable analyses are recommended to validate these findings.

CONCLUSION

In this survey of 205 people attending health facilities in four districts of Punjab, a history of vitamin B12 deficiency was reported by 72.2% and a diagnosis of peripheral neuropathy by 65.9%. Reported deficiency was strongly associated with metformin use, but so was the probability of knowing one's B12 status at all, and the second observation constrains how the first may be read. Testing in this setting appears to follow the prescription rather than the risk, which both inflates the measured association and leaves people with diabetes who are not taking metformin without assessment. A serum-based study in the same districts, recording metformin dose and duration and using a validated neuropathy instrument, would establish whether the burden suggested here is real.

Ethical Approval

The study was approved by the Research and Ethics Committee of Quaid-e-Azam College of Pharmacy, Sahiwal (Approval No. QACP/24/355).

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions

All authors contributed equally to the conceptualization, execution, and preparation of the present study. All authors reviewed and approved the final manuscript

Informed Consent
Informed consent was obtained from all participants before their enrollment in the study. The participants were adequately informed about the study objectives and procedures, and their privacy and confidentiality were ensured throughout the study.

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Conflict of Interest
The authors declare that they have no conflicts of interest related to the publication of this study.

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Declaration of AI-Assisted Language Editing
Grammarly AI was used for language and grammar correction, while Gemini AI was used for figure generation.

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