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CORRELATION BETWEEN DURATION OF METFORMIN USE AND VITAMIN B12 DEFICIENCY IN PATIENTS WITH TYPE 2 DIABETES

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Abstract

Objectives: *To determine the frequency of vitamin B12 deficiency in patients with type 2 diabetes mellitus receiving long-term metformin therapy.*

Study Design: *A Cross Sectional Study.*

Study Settings: *Medical Unit 2, Allama Iqbal Teaching Hospital, Dera Ghazi Khan, Pakistan.* ***Duration of Study:*** *June'2024 to November'2024.*

Data Collection: *A total of 222 patients aged 40–80 years with a diagnosis of T2DM and on metformin therapy for over one year were enrolled through non-probability consecutive sampling. Data on demographics, clinical history, metformin dose and duration, and comorbidities were collected. Serum vitamin B12 levels were measured; deficiency was defined as levels <125 pmol/L.*

Results: *Of 222 cases, 54.1% were female and rest 102(45.9%) were male subjects, 60.11 ± 12.26 years was their mean age; while 28.08 ± 6.26 kg/m² BMI, 10.59 ± 5.85 years for duration of diabetes, and 10.62 ± 5.09 years as metformin usage duration. Vitamin B12 deficiency was found in 16 patients (7.2%). No statistically significant association was observed between deficiency and age, gender, smoking status, comorbidities, or metformin dose/duration ($p > 0.05$).*

Conclusion: *Although the overall percentage was modest, 7.2% of diabetic patients on extended metformin therapy exhibited vitamin B12 deficiency, warranting clinical attention. While no strong associations with clinical variables were observed, routine screening for B12 levels may be helpful in preventing long-term health issues among patients in this population.*

INTRODUCTION

There is a growing global epidemic of type 2 diabetes mellitus, with prevalence rising steadily across all regions, with 463 million cases recorded in 2019 and estimates suggesting a surge to 700 million by 2045, highlighting a growing public health crisis. In Pakistan, the prevalence of diabetes is high, at 26.3%.[1] Metformin, a widely used medication for type 2 diabetes, can lead to vitamin B12 deficiency over time. The use of metformin has been linked to reduced absorption of vitamin B12 in the gut.[2-3]

Since vitamin B12 is vital for nerve health and repair, its deficiency may lead to peripheral neuropathy.[4-5] Patients often experience tingling, numbness, or pain in their hands and feet, symptoms that can interfere with daily life. If the deficiency is not addressed, these symptoms may worsen over time. This underscores the necessity of regular monitoring of vitamin B12 levels in at-risk individuals and providing supplements when needed to prevent long-term complications.[6-7]

Evidence from regional studies further demonstrates how prevalence can vary. Almatrafi et al[8] noted a 17.5% rate of B12 deficiency in a Saudi sample, whereas Ali et al[9] found a 10.71% rate in Pakistani patients. Notably, Al Quran et al[10] observed an exceptionally high prevalence of 48.9%. This wide range suggests that ethnicity, healthcare access, and nutritional patterns may significantly influence outcomes.

The rationale of this study is to assess the frequency of vitamin B12 deficiency in patients presenting with diabetes on treatment with metformin. It is clinically known that among diabetic patients, a shortage of vitamins from the body is common. Moreover, metformin induces vitamin B12 malabsorption, which may increase the risk of developing vitamin B-12 deficiency. However, in the literature, it has been noticed that the frequency of vitamin B12 deficiency is low in some studies while others reported high.

METHODOLOGY:

This cross-sectional study was carried out in the Medical Unit 2 of Allama Iqbal Teaching Hospital, DG Khan, over a duration of six months (June'2024 to November'2024), following approval of the study synopsis by the Ethical Committee (Refernce No: 85/DME/DGKMC, Dated: 23/05/2024). The target population included patients aged 40 to 80 years who had been diagnosed with diabetes mellitus for at least one year and were on metformin therapy for more than one year. Based on a previously reported prevalence of vitamin B12 deficiency of 17.5% in diabetic patients on metformin, the WHO sample size calculator was used to determine a sample size of 222, with a 95% confidence level and a 5% margin of error. Patients were recruited through non-probability consecutive sampling, ensuring that every eligible patient presenting to the study setting was enrolled until the required sample size was reached.

The individuals included in this trial were—age between 40 and 80 years, documented diabetes mellitus for more than one year, and current metformin use for over a year—were invited to participate. Exclusion criteria included any patient who refused informed consent and those who were taking multivitamin supplements (as verified through medical records). Before participation, detailed information about the study's objectives and procedures was provided, and written informed consent was obtained from each patient. The study received prior approval from the hospital's ethics committee, and all data were handled with strict adherence to ethical and confidentiality protocols.

After obtaining consent, participants' data were recorded on a predesigned proforma. Demographic details such as name, age, and gender were noted, along with height and weight measurements, which were used to calculate BMI. Clinical information, including blood sugar levels (BSR), duration of diabetes, metformin dosage and duration, smoking status, and any existing comorbidities (IHG, hypertension, liver disease and CKD), was retrieved from medical records or through patient interviews. The definitions for the co-morbidities are followed standard clinical and investigative criteria as outlined in the operational definitions. Participants were grouped based on their smoking history into current smokers (actively smoking), ex-smokers (ceased smoking for at least six months), and non-smokers (never smoked).

Once the patient information had been collected, a blood sample of approximately 5 mL was drawn from each participant by a trained phlebotomist using sterile technique. This sample was placed in an appropriate collection tube and sent to the hospital

laboratory for measurement of serum vitamin B12 levels. Patients with a serum vitamin B12 level below 125 pmol/L were classified as having vitamin B12 deficiency. Those diagnosed with deficiency were offered treatment and counseling according to the hospital's standard protocols.

SPSS version 26 was used for data entry and statistical analysis. Numerical variables like age, BMI, blood sugar, diabetes duration, metformin dosage and treatment length, and vitamin B12 levels were presented as mean ± standard deviation. The Shapiro-Wilk test was applied to check if data followed a normal distribution. Categorical variables—including gender, BMI groups, smoking history, comorbid conditions, and vitamin B12 status—were expressed in terms of frequency and percentage. To explore associations between vitamin B12 deficiency and other variables, data were stratified by age, sex, BMI, smoking, comorbidities, diabetes duration, and metformin use. The chi-square test was used to assess statistical differences between subgroups.

RESULTS:

The age distribution reveals that 48.2% of the patients were between 40–60 years, while 51.8% were aged 61–80 years, with a mean age of 60.11 ± 12.26 years. In terms of smoking status, 33.8% were non-smokers, 35.1% were current smokers, and 31.1% were ex-smokers. Gender-wise distribution showed that 45.9% were male and 54.1% were female. Body Mass Index (BMI) categorization showed that 9.5% were underweight, 25.7% had normal weight, 25.7% were overweight, and a significant 39.2% were obese. The mean BMI was calculated as 28.08 ± 6.26 kg/m^2. Clinical parameters revealed a mean blood sugar random (BSR) of 245.66 ± 30.0 mg/dL. The average duration of diabetes mellitus was 10.59 ± 5.85 years. Metformin was used with a mean dose of 1270.27 ± 573.88 mg, and its average duration of use was 10.62 ± 5.09 years. The mean serum Vitamin B12 level recorded was 207.22 ± 57.15 pmol/L.

Hypertension was the most frequently reported comorbidity, affecting 62.6% of the patients, while 37.4% had no history of hypertension. Chronic kidney disease (CKD) was present in 22.1% of the cases, whereas 77.9% did not report this condition. Ischemic heart disease (IHD) was observed in 32.4% of the participants, with the remaining 67.6% being free from IHD. Liver disease was the least prevalent among the documented conditions, affecting 12.6% of patients, and 87.4% reported no liver dysfunction. Our study summarizes the frequency of Vitamin B12 deficiency among diabetic patients receiving metformin therapy. Of the 222 patients, 16 (7.2%) were diagnosed with Vitamin B12 deficiency, whereas the majority, 206 (92.8%), had normal Vitamin B12 levels. This finding indicates that although the overall prevalence of deficiency is relatively low, a notable proportion still presents with suboptimal Vitamin B12 status.

The table 2 provides a stratified analysis of Vitamin B12 deficiency based on various demographic and clinical variables. Among patients aged 40–60 years, 10.3% had deficiency compared to 4.4% in the 61–80 age group (p=0.091). Female patients showed a slightly higher deficiency rate (9.2%) compared to males (4.9%), (p=0.221). Smoking status did not show a significant relationship with Vitamin B12 deficiency (p=0.375), although smokers (10.3%) had higher deficiency rates than non-smokers (6.7%) and ex-smokers (4.3%). Among hypertensive patients, 7.9% were deficient compared to 6.0% in non-hypertensives (p=0.598). Similarly, no significant associations were found for chronic kidney disease (p=0.769), ischemic heart disease (p=0.510), or liver disease (p=0.989), indicating that these comorbidities did not significantly affect the likelihood of Vitamin B12 deficiency in this population.

Table 1: Demographics And Clinical Profile(N=222)

Variables		Count	Percent
Age	40-60	107	48.2
	61-80	115	51.8
	Mean±SD	60.11±12.26	
Smoking	Non-smoker	75	33.8
	Smoker	78	35.1
	Ex-Smoker	69	31.1

Gender	Male	102	45.9
	Female	120	54.1
BMI	Underweight	21	9.5
	Normal weight	57	25.7
	Overweight	57	25.7
	Obese	87	39.2
	Mean±SD	28.08±6.26	
BSR (mg/dL)		245.66±30.0	
Duration of DM (years)		10.59±5.85	
Metformin Dose (mg)		1270.27±573.88	
Metformin Duration (years)		10.62±5.09	
Vitamin B12 (pmol/L)		207.22±57.15	

Table 2: Vitamin B12 Deficiency In Patients Presenting With Diabetes On Treatment With Metformin According To Various Effect Modifiers(N=222)

Variable		(Vitamin B12 Deficiency)	(No Deficiency)	Total	P-value
Age(years)	40-60	11 (10.3%)	96 (89.7%)	107 (100%)	0.091
	61-80	5 (4.4%)	109 (95.6%)	114 (100%)	
Gender	Male	5 (4.9%)	97 (95.1%)	102 (100%)	0.221
	Female	11 (9.2%)	109 (90.8%)	120 (100%)	
Smoking	Non-smoker	5 (6.7%)	70 (93.3%)	75 (100%)	0.375
	Smoker	8 (10.3%)	70 (89.7%)	78 (100%)	
	Ex-Smoker	3 (4.3%)	66 (95.7%)	69 (100%)	
Hypertension	Yes	11 (7.9%)	128 (92.1%)	139 (100%)	0.598
	No	5 (6.0%)	78 (94.0%)	83 (100%)	
CKD	Yes	4 (8.2%)	45 (91.8%)	49 (100%)	0.769
	No	12 (6.9%)	161 (93.1%)	173 (100%)	
IHD	Yes	4 (5.6%)	68 (94.4%)	72 (100%)	0.51
	No	12 (8.0%)	138 (92.0%)	150 (100%)	
Liver Disease	Yes	2 (7.1%)	26 (92.9%)	28 (100%)	0.989
	No	14 (7.2%)	180 (92.8%)	194 (100%)	

DISCUSSION:

Vitamin B12 deficiency is a well-recognized adverse effect associated with prolonged metformin therapy in patients with type 2 diabetes mellitus (T2DM). While metformin remains the first-line treatment for T2DM due to its efficacy and safety, studies have consistently shown its potential to reduce serum vitamin B12 levels, likely through interference with absorption in the terminal ileum. Given the high burden of diabetes in Pakistan and the widespread use of metformin, evaluating vitamin B12 status in this population holds significant clinical relevance.

In our study of 222 patients, the frequency of vitamin B12 deficiency was 7.2%. This prevalence is relatively lower than that reported in several regional and international studies. For example, Nguyen et al[11] and Huynh et al[12] in separate cross-sectional studies from Vietnam, both reported a deficiency rate of **18.6**% among metformin-treated T2DM patients. Sulaiman et al[13] in Libya reported a higher prevalence of **20.7**%, and Hallit et al[14] found a rate of **13.1**% among Lebanese patients using metformin. Similarly, Farooq et al[15] from India observed that B12 deficiency was significantly more common in metformin users compared to non-users. In Pakistan, Ramzan et al[16] reported a deficiency prevalence of **significantly higher** among those taking more than 2000 mg/day of metformin for more than four years. These

differences in prevalence could be attributed to various factors such as dietary patterns, baseline nutritional status, and healthcare access, which vary between populations.

Demographically, the mean age of participants in our study was 60.1 years, with over half aged above 60. This aligns with other studies such as Wanichtanom et al[17] and Atkinson et al[18] which included older diabetic populations. Our cohort showed a slight female predominance (54.1%), which is comparable to the gender distributions reported by Hallit et al[14] and Sulaiman et al[13] Obesity was common, with 39.2% of patients classified as obese—an important observation given the association between obesity and insulin resistance, which often necessitates higher and prolonged use of metformin.

Although prior studies such as Farooq et al[15] and Wanichtanom et al[17] reported significant associations between vitamin B12 deficiency and higher metformin doses or longer treatment durations, our study did not find statistically significant correlations between deficiency and variables such as age, gender, smoking status, metformin dose or duration, or comorbidities. This discrepancy may be due to the small number of deficient patients in our sample (n=16), which may have limited the power to detect such associations. Nonetheless, non-significant trends in our data still showed slightly higher deficiency rates among smokers, females, and those aged 40–60, warranting further investigation in larger studies.

Vitamin B12 malabsorption associated with metformin use is thought to result from its inhibition of calcium-dependent transport processes in the terminal ileum, where B12 absorption normally occurs. Pawlak (2017)[19] and Al Zoubi et al[20] discussed how metformin interferes with this mechanism, leading to gradual depletion of B12 stores, particularly in patients with poor dietary intake or increased demand. These findings support the biological plausibility of our results and highlight the importance of regular biochemical monitoring in patients on chronic metformin therapy.

Clinically, the consequences of B12 deficiency are concerning, particularly for diabetic patients who are already at risk for peripheral neuropathy. Farooq et al. demonstrated that low B12 levels were associated with a higher frequency of clinical neuropathy in metformin users. Atkinson et al. also highlighted the neurological implications of undetected B12 deficiency and emphasized the need for early screening protocols, particularly in those on higher doses and longer durations of therapy. Although our study did not include neurological assessment, the risk of misattributing B12-related neuropathy to diabetes alone remains an important clinical concern. This study provides valuable local evidence from Pakistan, where limited data exist on this topic. Strengths include a clearly defined population, adequate sample size, and use of standardized laboratory methods. However, limitations include the single-center design, lack of dietary assessment, and absence of neurological evaluation. These factors may affect generalizability and underestimate the true burden of deficiency.

CONCLUSION:

Prevalence of vitamin B12 deficiency among metformin-treated diabetic patients is lower than that observed in many regional and international studies, it still underscores the need for increased clinical awareness. Given the potential complications of untreated deficiency, particularly in older and long-term metformin users, we recommend periodic screening for vitamin B12 levels as part of routine diabetes management.

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