An Analytical Study Of Serum Cyanocobalamin Levels In Diabetic Patients On Metformin Therapy For More Than 6 Months

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KEYWORDS

clinical symptoms, cross-sectional study, cyanocobalamin, vitamin b12 deficiency, metformin, type 2 diabetes mellitus.

ABSTRACT

Introduction: Type 2 diabetes mellitus (T2DM) is a prevalent metabolic disorder characterized by insulin resistance and hyperglycemia. Metformin, a common first-line treatment for T2DM, is associated with decreased serum vitamin B12 levels, potentially leading to deficiency and associated neurological and hematological complications. This study aimed to assess the prevalence of serum cyanocobalamin (Vitamin B12) deficiency in T2DM patients on metformin therapy for over six months and identify associated risk factors. **Methodology:** This cross-sectional analytical study was conducted at Krishna Vishwa Vidyapeeth, Karad, Maharashtra, over 18 months. Seventy T2DM patients on metformin for more than six months were included, excluding those with purely vegetarian diets, chronic alcoholism, or other significant comorbidities. Data collection involved fasting and postprandial blood sugar levels, HbA1c, and serum cyanocobalamin levels. Statistical analysis was performed using SPSS, with chi-square and t-tests applied for association and comparison analyses. **Results:** The study found that 27.14% of the patients had deficient serum cyanocobalamin levels (<200 pg/mL). A significant association was observed between longer duration of metformin therapy and lower cyanocobalamin levels ($p = 0.009$), and higher daily doses of metformin correlated with increased deficiency ($p = 0.017$). Clinical symptoms like fatigue, tingling sensations, and gastrointestinal disturbances were more prevalent in patients with cyanocobalamin deficiency ($p < 0.05$). **Conclusion:** The findings highlight a notable prevalence of vitamin B12 deficiency among T2DM patients on metformin, particularly with prolonged and higher dose use. Regular monitoring of cyanocobalamin levels is crucial to prevent and manage deficiency-related complications in this population.
Categories: Nutrition, Geriatrics, Internal Medicine
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Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. [1] Type 2 diabetes mellitus (T2DM) is the most common prevalent form, which accounts for approximately 90-95% of all diabetes cases globally [2]. India has seen a rapid rise in diabetes prevalence, with an estimated 77 million adults are living with diabetes, positioning the country as the diabetes capital of the world [3].

Metformin, a biguanide, is widely regarded as the first-line pharmacotherapy for T2DM due to its efficacy, safety profile, and cost-effectiveness. It acts primarily by reducing hepatic glucose production and improving insulin sensitivity. However, chronic use of metformin causes a decrease in serum vitamin B12 (cobalamin) levels, potentially leading to deficiency and subsequent neurological and hematological complications [4].

Cyanocobalamin is an essential water-soluble vitamin involved in most of the critical biological processes. It is obtained from sources such as meat, dairy products, and fortified cereals. Deficiency in cyanocobalamin can lead to megaloblastic anemia, neuropathy, and cognitive disturbances [5]. The mechanisms underlying metformin-induced cyanocobalamin deficiency are due to faulty absorption process, due to changes in gut microbiota and intrinsic factor secretion [6].

Several studies have reported a prevalence of cyanocobalamin deficiency among metformin users ranging from 5.8% to 33% [7,8]. This wide variability highlights the need for region-specific data. In India, where vegetarianism is prevalent, the risk of cyanocobalamin deficiency may be further compounded by metformin use, emphasizing the necessity for focused research in this area [8].

The clinical implications of cyanocobalamin deficiency are profound, particularly in type 2 diabetic patients who are already at risk of neuropathy. Diabetic peripheral neuropathy (DPN) is a common and debilitating complication of diabetes, characterized by pain, numbness, and tingling in the extremities. It has always been suggested that cyanocobalamin deficiency may exacerbate DPN symptoms or even be misdiagnosed as diabetic neuropathy, leading to suboptimal management [9]. Early detection and correction of cyanocobalamin deficiency in diabetic patients on metformin could potentially improve clinical outcomes and quality of life.

Given the growing burden of diabetes in India and the widespread use of metformin, it is crucial to evaluate serum cyanocobalamin levels in this population. This study aims to assess the prevalence of serum cyanocobalamin deficiency in diabetic patients on treatment with metformin for more than six months and to identify risk factors associated with this deficiency. By providing region-specific data, this study seeks to inform clinical practice and guide the management of metformin-treated diabetic patients in India [7,8].

To achieve these objectives, the study will involve a cross-sectional analysis of serum cyanocobalamin levels in diabetic patients receiving metformin therapy for more than six months. The study population will include individuals from diverse socioeconomic backgrounds and dietary habits to ensure comprehensive representation. Additionally, potential confounding factors such as age, gender, duration of diabetes, and dietary intake will be considered in the analysis to provide a nuanced understanding of the association between metformin use and cyanocobalamin levels [8].

Our study will address a critical gap in the literature by providing evidence on the prevalence and determinants of cyanocobalamin deficiency in Indian diabetic patients on long-term metformin therapy. The findings are expected to have noteworthy implications for clinical practice, potentially leading to improved screening, prevention, and management strategies for cyanocobalamin deficiency in this vulnerable population.

Materials And Methods

The study was a single-center, hospital-based cross-sectional analytical study conducted at the Department of General Medicine, Krishna Vishwa Vidyapeeth, Karad, Maharashtra, over 18 months from September 2022 to March 2024. It included patients with Type 2 Diabetes Mellitus (T2DM) who had been on metformin therapy for more than six months, irrespective of their HbA1c levels. The exclusion criteria were patients with a purely vegetarian diet, chronic alcoholism, active cardiac, renal, bone, or muscle disorders, severe anemia, a history of gastric surgery, and those using oral hypoglycemics other than metformin.

Data collection involved enrolling patients who met the inclusion criteria, administering a questionnaire about daily symptoms, and conducting investigations, including fasting and postprandial blood sugar levels, HbA1c levels, and serum cyanocobalamin levels. The sample size was calculated using a prevalence rate of 8.9% for Vitamin B12 deficiency in T2DM patients on metformin, as reported by Ting RZ et al., with a precision of 0.07 and a z-statistic of 1.96. This calculation resulted in a minimum sample size of 63, with an additional allowance for a 10% dropout rate, bringing the total to 70 cases using formula for sample size calculation n = 4pq/ d2 [10].

Statistical analysis was performed using frequencies and percentages for qualitative data, along with charts and graphs. Quantitative data were analyzed for mean and standard deviation. The study utilized the Chi- square test for associations and the unpaired t-test for comparisons between quantitative variables.

Correlations between serum cyanocobalamin deficiency and diabetes severity (measured by HbA1c levels) were evaluated. A p-value of less than 0.05 was considered statistically significant at a 95% confidence interval.

Ethical clearance was obtained from the institutional ethics committee with approval letter number: 352/2021-2022

Written informed consent was taken from all the participants / relatives before their enrolment in the study.

Results

We studied 70 diabetes patients on metformin therapy for more than 6 months. It was observed that maximum number of subjects 24 (34.29%) were in age group 51-60 years followed by 61-70 years (32.86%). The Mean age was 59.53 ±10.41 years. The maximum number of subjects 41 (58.57%) were males followed by 29 females (41.43%). Maximum number of subjects 28 (40%) had diabetes for 1-5 years. The

Mean diabetes duration was 4.40 ± 2.81 years. The mean fasting blood sugar level among subjects was 196.93

± 57.11 mg/dl. The mean post prandial blood sugar level among subjects was 306.07 ± 90.44 mg/dl while mean HbA1c was 8.09 ± 1.21.

Among the 70 patients, 25 had been on Metformin for 1-5 years (35.71%), making this the most common duration group. A slightly smaller group of 23 patients had been treated for 6-10 years (32.86%).

Additionally, 18 patients had a treatment duration of 10-15 years (25.71%), while a minority of 4 patients had been on Metformin for more than 15 years (5.71%). This distribution indicates that the majority of the participants had been undergoing Metformin treatment for less than 10 years, highlighting a potential trend in treatment duration among the study population. [Fig 1]

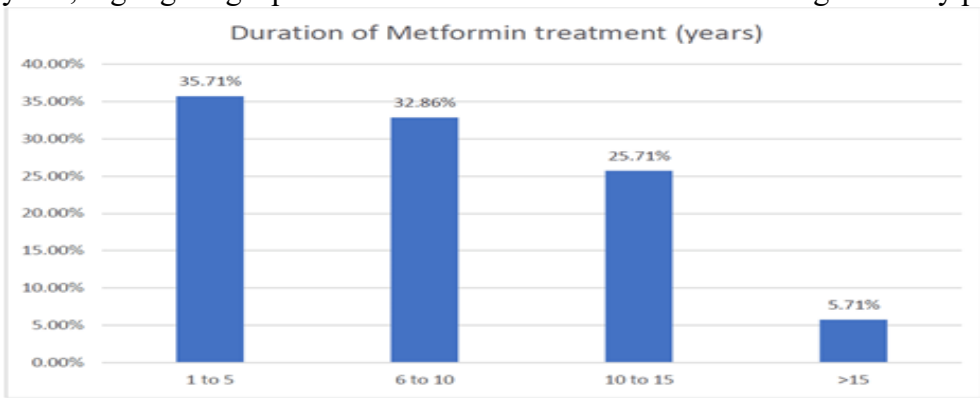

FIGURE 1: Distribution according to duration of Metformin treatment

Table 1 details the prevalence of serum cyanocobalamin (vitamin B12) levels among the 70 patients in the study. A majority of 51 patients (72.86%) had normal serum cyanocobalamin levels, ranging from 200 to 900 pg/mL. In contrast, 19 patients (27.14%) were found to have deficient levels, defined as less than 200 pg/mL. This indicates that while most patients maintained adequate vitamin B12 levels, a significant proportion exhibited deficiency, which may have clinical implications for their overall health and response to treatments. [Table 1]

Serum Cyanocobalamin Levels	No of patients	Percentage
Normal (200 to 900)	51	72.86%
Deficient (<200)	19	27.14%
Total	70	100%

TABLE 1: Distribution according to prevalence of Serum Cyanocobalamin Levels

Table 2 describes the duration of metformin treatment and serum cyanocobalamin levels in diabetic patients. The mean duration of metformin treatment for patients with deficient serum cyanocobalamin levels (n=19) was 6.13 years with a standard deviation of 2.12 years. In contrast, the mean duration for patients with normal levels (n=51) was 3.43 years with a standard deviation of 2.03 years. The difference in the mean duration of metformin treatment between the two groups was statistically significant, with a p- value of 0.009. This suggests that a longer duration of metformin treatment is associated with a higher likelihood of serum cyanocobalamin deficiency in diabetic patients. [Table 2]

Duration of metformin treatment (years)	Serum Cyanocobalamin Levels		P value
	Deficient (n=19)	Normal (n=51)	
Mean duration	6.13 ± 2.12	3.43 ± 2.03	t = 4.79, p = 0.009 (S)

TABLE 2: Relation of Duration of Metformin Treatment and Serum Cyanocobalamin Levels in diabetic patients

In Table *3*, the daily dose of metformin and serum cyanocobalamin levels in diabetic patients is analyzed. Among those taking 500 mg/day of metformin, 1 patient (5.26% of the 19 deficient cases) had deficient serum cyanocobalamin levels, while 18 (94.74% of the 51 normal cases) had normal levels. For patients on 850 mg/day, 4 (21.05% of the deficient cases) had deficient levels and 13 (78.95% of the normal cases) had normal levels. Among patients taking 1000 mg/day or more, 14 (73.68% of the deficient cases) had deficient levels and 20 (26.32% of the normal cases) had normal levels. The differences were statistically significant, with a p-value of 0.017, indicating that higher daily doses of metformin (1000 mg/day or more) correlate with an increased likelihood of serum cyanocobalamin deficiency in diabetic patients [Table *3*].

Dose of metformin (mg/day)	Serum Cyanocobalamin Levels Deficient (n=19)	Serum Cyanocobalamin Levels Normal (n=51)
500 mg/day	01	18
850 mg/day	04	13
1000 mg/day or more	14	20
X2 = 8.09, df = 2, P = 0.017, Significant		

TABLE 3: Relation of Dose of Metformin (Mg)/Day And Serum Cyanocobalamin Levels

Table *4* examines the distribution of clinical manifestations among diabetic patients on metformin therapy, categorized by serum cyanocobalamin levels (deficient vs. normal). Among the 19 patients with deficient serum cyanocobalamin levels, 10 (52.63%) experienced fatigue

or lethargy, which was significantly higher compared to 6 (11.76%) in the normal group ($p < 0.001$). Behavioral changes or confusion were reported in 3 (15.79%) patients with deficient levels, contrasting with none (0%) in the normal group ($p = 0.004$).
Recurrent mouth ulcers were prevalent in 10 (52.63%) patients with deficient levels and 1 (1.96%) patient with normal levels ($p < 0.001$). Gastrointestinal disturbances were noted in 4 (21.05%) patients with deficient levels and 2 (3.92%) patients with normal levels ($p = 0.022$). Tingling sensation in the feet and palms was reported by 12 (63.16%) patients with deficient levels and 13(25.49%) patient with normal levels ($p < 0.001$). Similarly, visual disturbances were observed in 4 (21.05%) patients with deficient levels and 1 (1.96%) patient with normal levels ($p = 0.006$). These findings highlight significant associations between serum cyanocobalamin deficiency and various clinical symptoms among patients on metformin therapy. [Table *4*]

Clinical Symptoms	**Serum Cyanocobalamin Levels**		**P Value**
	Deficient (n=19)	Normal (n=51)	
Fatigue, Lethargy	10	06	<0.001*
Behavioral Changes Confusion	3	0	0.004*
Recurrent Mouth Ulcers	10	1	<0.001*
Gastrointestinal Disturbances	4	2	0.022*
Tingling Sensation of Feet And Palm	12	13	<0.001*
Visual Disturbances	4	1	0.006*

TABLE 4: Distribution According to Clinical Manifestations of Serum Cyanocobalamin Deficiency In Patients On Metformin Therapy

FIGURE.2 explores the relationship between serum cyanocobalamin levels (deficient vs. normal) and specific clinical signs among diabetic patients. Among the 19 patients with deficient serum cyanocobalamin levels, 4 (21.05%) exhibited pallor, compared to 6 (11.76%) in the normal group, and this difference was not statistically significant ($p = 0.32$). Knuckle pigmentation was significantly more prevalent in patients with deficient levels, with 12 (63.16%) affected, compared to 3 (5.88%) in the normal group ($p < 0.001$). Similarly, bald tongue or red beefy tongue was observed in 14 (73.68%) patients with deficient levels, while none (0%) in the normal group exhibited these signs ($p < 0.001$). These findings underscore significant associations between serum cyanocobalamin deficiency and specific clinical signs among diabetic patients on metformin therapy.

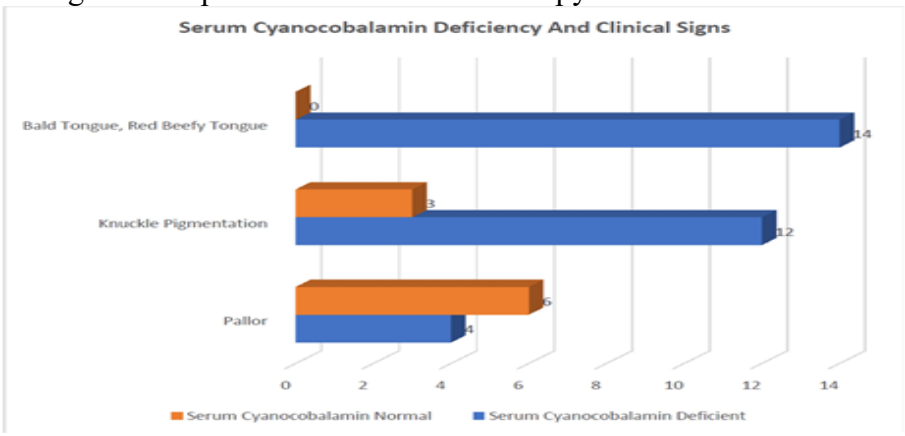

FIGURE 2: Relation Of Serum Cyanocobalamin Deficiency and Signs in Patients On Metformin Therapy

Discussion

In this analytical study, we examined serum cyanocobalamin (Vitamin B12) levels in diabetic patients who had been on metformin therapy for more than six months, aiming to understand the prevalence and contributing factors of serum cyanocobalamin deficiency in this population. We observed that mean age of participants was 59.53 ± 10.41 years, a study by Singh et al. found similar age distribution among diabetic patients on metformin therapy in India, emphasizing the prevalence of diabetes in this age group [11].

Additionally, Zhang et al. also reported a high prevalence of diabetes among individuals aged 50-70 years in their study. [12]

We had 58.57% males and 41.43% females, indicating a higher prevalence of diabetes among males. A study by Rojas et al., who also reported a higher prevalence of diabetes in males compared to females. [13]

The majority of the patients (40%) had been diagnosed with diabetes for 1-5 years, with a mean duration of

6.40 ± 2.81 years. This finding is consistent with the study by Kumar et al., which found a significant proportion of patients with a shorter duration of diabetes on metformin therapy [14]. Similarly, a study by Bailey et al. highlighted that the duration of diabetes significantly impacts the management and progression of the disease [15].

The mean fasting blood sugar (FBS) level was 196.93 ± 57.11 mg/dl, the mean postprandial blood sugar (PPBS) level was 306.07 ± 90.44 mg/dl, and the mean HbA1c was 8.09 ± 1.21. These elevated levels indicate poor glycemic control among the study participants. A study by Adler et al. reported similar findings, emphasizing the challenges in achieving optimal glycemic control in diabetic patients [16]. In the Indian context, Mohan et al. also highlighted poor glycemic control among diabetic patients, underscoring the need for improved management strategies [17].

Most patients had been on metformin therapy for 1-5 years (35.71%), followed by those on treatment for 6- 10 years (32.86%). This pattern is similar to that reported by DeFronzo et al., who found a significant proportion of patients on metformin for less than 10 years [18]. An Indian study by Gupta et al. also found similar treatment durations among diabetic patients, highlighting the widespread use of metformin as a first-line therapy [19].

The study found that 27.14% of patients had deficient serum cyanocobalamin levels (<200 pg/mL). This prevalence is consistent with findings by Aroda et al., who reported a significant proportion of metformin- treated patients with Serum Cyanocobalamin deficiency [20]. In the Indian context, a study by Reinstatler et al. also highlighted the high prevalence of Serum Cyanocobalamin deficiency among diabetic patients on long-term metformin therapy [21].

A significantly longer duration of metformin treatment was associated with serum cyanocobalamin deficiency ($p = 0.009$). This finding is consistent with studies by Ting et al. and an Indian study by Singla et al., which reported a similar association between prolonged metformin use and Serum Cyanocobalamin deficiency [22, 23]. These results underline the importance of monitoring Serum Cyanocobalamin levels in patients on long-term metformin therapy to prevent deficiency-related complications.

Higher doses of metformin (1000 mg/day or more) were significantly associated with serum cyanocobalamin deficiency ($p = 0.017$). This finding aligns with studies by Bauman et al. and Kalra et al., which also reported a dose-dependent relationship between metformin use and Serum Cyanocobalamin deficiency [24, 25].

These results highlight the need for careful dosage management and monitoring of Serum Cyanocobalamin levels in patients on high-dose metformin therapy.

Patients with deficient serum cyanocobalamin levels exhibited significantly higher rates of clinical symptoms such as fatigue, behavioral changes, recurrent mouth ulcers, gastrointestinal disturbances, tingling sensations, and visual disturbances ($p < 0.05$). Studies by Obeid et al.

and Singh et al. have reported similar associations between Serum Cyanocobalamin deficiency and these clinical manifestations [26, 27]. These findings underscore the clinical relevance of monitoring and addressing Serum Cyanocobalamin deficiency in patients on metformin therapy to improve their overall quality of life.

Patients with deficient serum cyanocobalamin levels had significantly higher prevalence of clinical signs such as knuckle pigmentation and bald/red beefy tongue ($p < 0.001$). Similar findings were reported by Adams et al. and an Indian study by Sharma et al., highlighting the importance of these clinical signs as indicators of Serum Cyanocobalamin deficiency [28, 29]. Our study results emphasize the need for regular clinical assessments to detect and manage Serum Cyanocobalamin deficiency in patients on long-term metformin therapy.

Conclusions

This study elucidates the significant prevalence of vitamin B12 deficiency among type 2 diabetes mellitus patients on metformin therapy, particularly with prolonged use and higher doses. Our findings reveal that 27.14% of patients exhibited deficient serum cyanocobalamin levels, with a significant association between longer duration of metformin treatment and lower cyanocobalamin levels ($p < 0.05$), and higher daily doses of metformin correlated with increased cyanocobalamin deficiency ($p < 0.05$). Clinical manifestations such as fatigue, behavioral changes, mouth ulcers, gastrointestinal disturbances, tingling sensation, and visual disturbances were significantly more common in patients with cyanocobalamin deficiency ($p<0.05$).

Additionally, specific clinical signs like knuckle pigmentation and a bald or red beefy tongue were significantly associated with cyanocobalamin deficiency ($p<0.001$). These findings underscore the necessity for regular monitoring of vitamin cyanocobalamin levels in diabetic patients on metformin, especially those on long-term or high-dose regimens, to prevent and manage potential deficiencies effectively.

Additional Information

Disclosures

Human subjects: Consent for treatment and open access publication was obtained or waived by all participants in this study. Institutional Ethics Committee issued approval 352/2021-2022. The Institutional Ethics Committee has hereby given permission to initiate the research project (Protocol Number 352/2021- 2022) titled, "STUDY OF CYANOCOBALAMIN LEVELS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS ON
METFORMIN THERAPY FOR MORE THAN 6 MONTHS" by Dr. Mayuresh Govind Kale under the guidance of Dr. Anil Bhattad, Assistant Professor, Department of Medicine, Krishna Institute of Medical Sciences, Krishna Institute of Medical Sciences "Deemed To Be University", Karad. Animal subjects: All authors have confirmed that this study did not involve animal subjects or tissue. Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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