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Original Research Article

Correlation and Association of Serum B12 Levels in Type-2 Diabetes Mellitus Patients Treated with Metformin: A Study Conducted at a Tertiary Care Hospital of Gujarat

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ABSTRACT

Background: Type 2 Diabetes mellitus(T2DM) is the most common endocrine disorder, affecting millions of people, leading to morbidity and mortality. Metformin is most widely used antidiabetic drug due to various reasons. Long-term metformin treatment in type 2 diabetes mellitus patients can cause vitamin B12 insufficiency. Severe deficits, if untreated, may lead to life-threatening neurological issues, which are often overlooked and undertreated.

Objective: To analyze the correlation between the duration and dosage of metformin treatment of more than one year in T2DM Patient and their combined impact on serum B12 levels among T2DM patients.

Material and methods: Hospital-based Cross-Sectional observational study evaluated 42 diabetic subjects, encompassing both sexes aged 18 to 70 years, who had been on metformin medication for more than one year. Assessment involved a comprehensive questionnaire and measurements of FBS, HbA1c, and serum B12 levels. Additionally, data on Metformin Dosage and Duration of its use were collected. B12 deficiency was defined as serum B12 levels ≤ 200 pg/ml. Data analysis was conducted by statistically comparing the mean vitamin B12 levels in each group using the One-Way ANOVA test through SPSS version 23 software.

Results: Increase in the duration of metformin drug usage correlates with a significant decrease in serum vitamin B12 levels, as confirmed by ANOVA test results ($p < 0.05$).

Conclusion: This study finding indicate that vitamin B12 deficiency is linked to a higher risk of worsening diabetic neuropathy. Therefore, individuals with diabetes who take higher doses of metformin (over 1000mg) for extended periods (≥ 8 years) should undergo regular monitoring of their vitamin B12 levels. Proactive use of vitamin B12 supplementation, whether for prevention or treatment, can help mitigate this concern.

Keywords: Metformin, Type 2 Diabetes Mellitus, Vitamin B12 levels, Fasting Blood Glucose, Glycated Hemoglobin

INTRODUCTION

Metformin stands as a cornerstone in Managing type 2 diabetes mellitus due to its affordability, effectiveness, and favorable impact on body weight. Its well-tolerated side effect profile further solidifies its widespread adoption in clinical practice. Vitamin B12 that contains cobalt and functions as a co-factor for enzymes that are important for metabolism [1].

Several physiologically active forms of vitamin B12 are produced intracellularly including adenosyl-Cbl, methyl cobalamin and vitamin B12. [2-4] According to a recent report by the World Health Organization (WHO), India has surpassed China and now has the highest number of diabetics in the world.
Approximately 77 million of total population are affected by diabetes mellitus in India between the ages of 20 and 79, which accounts 8.9% of the population. [5,6] A chronic metabolic disorder, diabetes mellitus which is characterized as elevated levels of blood glucose, caused by relative or an absolute-insulin insufficiency, insulin resistance (IR) due to dysfunctional cells. Diabetes is classified into an early and late onset auto-immune forms, namely T1D and T2D respectively.

Although, T2D is usually used to describe one of the types of diabetes which is not auto-immune or monogenic in origin, and it has become more largely known that it reflects several pathophysiological states.[7] Decreased insulin production is a peculiar feature of T1D, which otherwise is referred as insulin-dependent, childhood/ juvenile-onset which demands regular insulin therapy. Alternatively, the body's ineffective use of insulin leads to T2D. It is also called as non-insulin-dependent or adult-onset diabetes. More than 95% affected by Type 2 DM having the primarily cause of decreased physical activity and high total body mass (TBM) index.[8]
Metformin, a biguanide-class medication, is an anti-hyperglycemic agent and the cornerstone of type 2 diabetes mellitus (T2DM) treatment, decreasing blood glucose levels and insulin resistance by enhancing insulin sensitivity.[9]

The data from the Indian Comprehensive National Nutrition Survey (CNNS) was analyzed, and the findings, published in the August 2023 edition of the National Library of Sciences journal, revealed concerning trends. Many States like Gujarat and Punjab has majorly recorded vitamin B12 Deficiency patient, while kerala & west Bengal contributes the least in this disease. Teenagers, Particularly in Gujarat, are notable vulnerable, with 47.6% experiencing B12 deficiency. Recent reports over the prevalence of vitamin B12 deficiency in India ranges from 12% to 67%. [10,11]

This study was conducted to further explore this question This study investigates the impact of metformin on serum vitamin B12 levels in patients with Type 2 diabetes mellitus, with a focus on analyzing the effects of metformin dosage and duration on these levels. The research aims to delve deeper into this important question.

MATERIAL AND METHODS

A hospital-based cross-sectional observational study was conducted at a tertiary care hospital in Gujarat, Undertaken within the Department of Biochemistry from April 2024 to July 2024, following approval from the Institutional Ethical Committee, ethical considerations were ensured through informed consent procedures. Forty-two patients were selected from the hospital biochemistry laboratory using a systematic sampling approach after rigorous screening. Patients who approved the criteria of the American Diabetic Association were defined as type 2 diabetes patients. [12]

Type 2 diabetes was diagnosed as follows:
Patients were defined as having type 2 diabetes if they met the American Diabetes Association criteria:
Fasting blood sugar > 126 mg/dL, or
Blood sugar > 200 mg/dL, 2 hours after glucose challenge.
Additionally, someone with symptoms of diabetes and an HbA1c ≥6.5% (≥48 mmol/mol) on a single occasion confirms the diagnosis of diabetes. In cases where there are no symptoms, it is recommended to repeat the HbA1c test after two weeks to eliminate the possibility of laboratory errors. If the repeat HbA1c test also yields a result of 6.5% (48 mmol/mol) or higher, the diagnosis can be confirmed.

Vitamin B12 deficiency in blood serum was diagnosed as follows:
B12 deficiency is defined as having a blood level of vitamin B12 below 200 picograms per milliliter (pg/mL) or 148 picomoles per liter (pmol/L)[13].

The inclusion criteria for patients are as follows:

Patient of (T2DM) aged between 18 to 70 years old.
Patients who have been undergoing metformin treatment for a minimum of one year.
Type 2 diabetes Mellitus patients selected as per the above-mentioned diagnosis criteria.

The exclusion criteria for patients are as follows:

Patients who were pregnant or had history of diseases like pernicious anemia, malabsorption syndrome, Autoimmune thyroid disease, Chronic hepatitis, chronic kidney disease stage 4 and above. Others who were chronic alcohol users and smokers. Beside this subjects who had been consuming oral/parenteral Vitamin B12 or multivitamin supplements. Patients with Recall biased error was also excluded from this study.

Patients with type 1 diabetes mellitus under insulin treatment.

The "Metformin" group included patients with type 2 diabetes mellitus who had been treated with metformin for ≥12 months. Patients who avoided eating eggs or any form of meat were classified as vegetarians, while those who had meat or eggs were classified as non-vegetarians. Patient history regarding the onset of diabetes, metformin dosage and duration, along with recent (within the last three months) measurements of glycosylated hemoglobin (HbA1c), S. vitamin B12 levels, fasting blood sugar and Random blood sugar levels were extracted from the previous records of Hospital, patient prescriptions, and medications were reviewed to identify any Vitamin B12-containing supplements. Also, patients who had shown a list of commonly available multi-vitamins that include vitamin B12, were asked if they had used any of these in the past.

Blood samples were collected, Followed by serum storage at 4-8°C for later analysis. Total Serum Vitamin B12 levels were measured using a solid-phase chemiluminescence immunoassay (CLIA) on a MAGLUMI 800 analyzer with commercial kits from Maglumi. Deficiency of Vitamin B12 was defined as levels below 200 pg/ml. In clinical studies, 95% of individual results ranged between 200-1100 pg/ml.

The MINDRAY-200 (GOD-POD method) assay was employed to measure fasting Blood glucose and Random Blood Glucose levels, with WHO criteria identifying levels above 126 mg/dl as indicative of diabetes. In this method, glucose is oxidized by enzyme, glucose oxidase (GOD) to produce hydrogen peroxide (H2O2). Peroxidase (POD) then catalyzes the reaction of H2O2 with 4-aminoantipyrine and p-hydroxybenzoic acid sodium, resulting in the formation of a colored quinoneimine dye. The increase in absorbance of this dye is directly proportional to the glucose concentration.

The Afinion HbA1c assay by Abbott is a fully automated boronate affinity test used to measure the percentage of hemoglobin A1C in human whole blood.

Statistical analysis was conducted using SPSS version 23. Continuous variables were expressed as mean ± standard deviation, while categorical variables were represented as percentages or proportions. The comparison of Vitamin B12 levels among different metformin duration groups used One-way ANOVA test for continuous variables, and among metformin dosage groups, Student's t-test was used. Scatter plot and linear regression analyses were conducted to examine the effect of metformin duration and dosage on S. vitamin B12 levels among patients with type 2 diabetes mellitus.

RESULTS

A study followed forty-two diabetes patients to compare Vitamin B12 levels among different metformin duration groups and dosage groups.

In the study, Table-1 Shows the mean age of the total population was 51.88 ± 10.36 years. The mean ± SD age of the female participants was 47.18 ± 8.23 years, while the mean ± SD age of the male participants was 54.76 ± 10.62 years.

Twenty patients belonged to Group A (patients on metformin with a dosage of less than 1000 mg), and twenty-two patients belonged to Group B (patients on metformin with a dosage of more than 1000 mg). Additionally, the patients were categorized into four groups based on the duration of metformin use: four patients belonged to Group A (patients on metformin for less than 2 years), twelve patients belonged to Group B (patients on metformin for 2-5 years), fourteen patients belonged to Group C (patients who were on metformin for 5-8 years), and twelve patients belonged to Group D (patients on metformin for ≥ 8 years).

Table 2 presents a comparative analysis of Vitamin B12 levels across various metformin usage duration groups. The data indicate that the highest mean Vitamin B12 levels were observed in the group with metformin usage duration of less than two years. In contrast, the group with usage duration of eight years or more shows the lowest mean Vitamin B12 levels. One-way ANOVA statistical analysis yielded a significant p-value ($p<0.05$) for Vitamin B12 levels, highlighting the effect of metformin duration on serum Vitamin B12 concentrations.

Chart 1 illustrates that Vitamin B12 levels are highest in the metformin duration group of less than 2 years and lowest in the group with duration of 8 years or more. Consequently, as the duration of metformin use increases, a deficiency in serum Vitamin B12 levels (pg/mL) is observed in diabetes mellitus patients. This result is significant ($p<0.05$).

Therefore, as the duration of dose increases, we observed deficiency of serum vitamin B12 levels (pg/mL) in the diabetes mellitus patients.

The chi-square test revealed a significant association between Metformin use duration and Vitamin B12 deficiency, particularly in the 5-8 years group, while post-hoc Tukey's HSD analysis indicated significant differences in Vitamin B12 levels between the ≤2 years and 5-8 years groups (p = 0.0056), ≤2 years and ≥8 years groups (p = 0.0007), 2-5 years and 5-8 years groups (p = 0.0010), and 2-5 years and ≥8 years groups (p = 0.0001).

Table 3 presents a comparison of Vitamin B12 levels among patients grouped by metformin dosage. Notably, the mean value of Vitamin B12 appears higher in patients receiving <1000 mg of metformin compared to those receiving >1000 mg. Statistical analysis utilizing the Student's t-test yielded a non-significant p-value for Vitamin B12 (p>0.05).
Figure 2 further illustrates this trend, with the highest percentage of Vitamin B12 values observed in the <1000 mg metformin dosage group and the lowest in the >1000 mg group. Consequently, it is evident from the graph that as the metformin dosage increases, Vitamin B12 levels tend to decrease, albeit the disparity between these two groups is statistically non-significant (p>0.05).

Therefore, from the given figure, it is clear that as the dose increases, a vitamin B12 level becomes deficient. But the difference between these two groups is statistically non-significant. (p>0.05)
One-way ANOVA statistical analysis yielded a significant p- value (p<0.05) for Vitamin B12 levels, highlighting the effect of metformin duration on serum Vitamin B12 concentrations.

Table-1: Age Distribution of Total Population and by Gender

Population	Mean Age (Year) ± SD
Total	51.88±10.36
Female	47.18±8.23
Male	54.76±10.62

Table-2: Comparison of Vitamin B12 levels among metformin duration groups

Metformin Duration (Years)	No.of patients	Mean ± SD Vitamin B12 levels (pg/mL)	P-value
< 2	4	210.25±16.33	<0.05(s)
2-5	12	203.25±58.71	
5-8	14	136.85±41.39	
≥8	12	116.36±37.08	
Total	42	158.02±58.27	

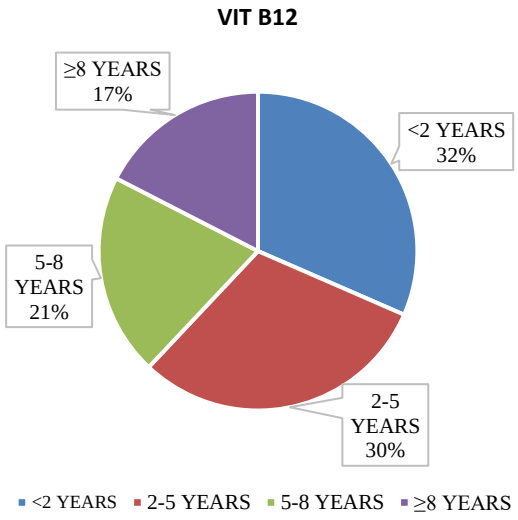

Figure-1: Values of vitamin B12 with Metformin duration

The Student's t-test statistical analysis yielded a non-significant p-value (p > 0.05) for the effect of metformin dosage on vitamin B12 levels.

Table-3: Comparison of Vitamin B12 levels among metformin dosage groups.

Metformin Dose(mg)	No.of patient	Mean±SD Vitamin B12 (pg/mL)	P-VALUE
< 1000	20	194.47±54.55	>0.05(s)
> 1000	22	126.55±40.97	

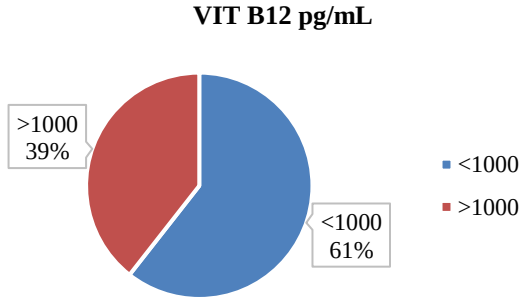

Figure-2: Percentage values of vitamin B12 as per dose of Metformin

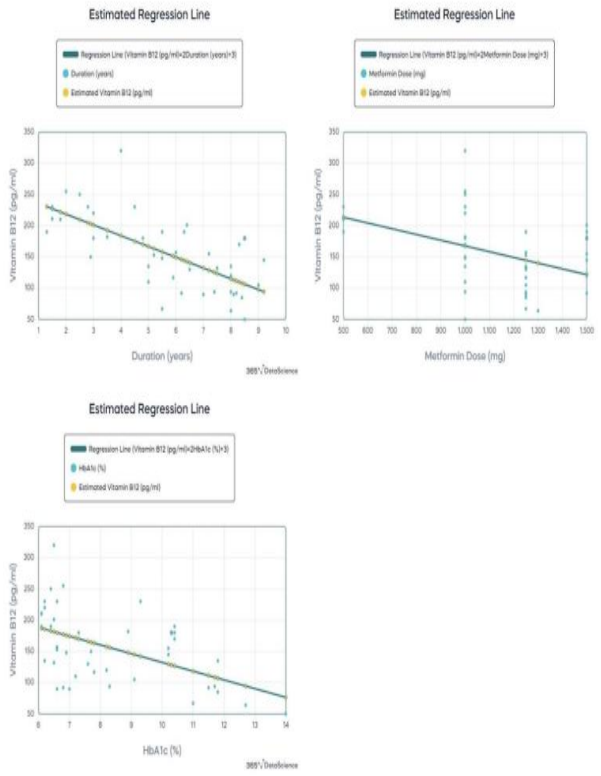

Figure-3: Relationships between serum vitamin B12 levels and the dosage of metformin, the duration of metformin therapy, and HbA1c levels, respectively

DISCUSSION

In our cross-sectional observational study, we examined 42 patients with type 2 diabetes mellitus to assess the effect of metformin Duration and Dosage on S. vitamin B12 levels. Our findings indicated a significant inverse relationship between metformin dosage (mg) and duration of use (years) with vitamin B12 levels. Notably, patients who had been taking metformin for ≥ 8 years exhibited the lowest vitamin B12 levels, with a mean of 116.36 ± 37.08 pg/mL.

The p-value for the duration of metformin use (years) was <0.05, indicating statistical significance. Although the p-value for the dosage of metformin (mg) was >0.05, which is not statistically significant, our study clearly shows that as the dosage increases, vitamin B12 levels decrease in serum.

Post-hoc Tukey's HSD analysis showed significant differences in Vitamin B12 levels among different Metformin use groups. Specifically, there were notable differences between the ≤2 years and 5-8 years groups (p = 0.0056, df = 38), the ≤2 years and ≥8 years groups (p = 0.0007, df = 38), the 2-5 years and 5-8 years groups (p = 0.0010, df = 30), and the 2-5 years and ≥8 years groups (p = 0.0001, df = 30).

These results highlight that longer Metformin use is associated with lower Vitamin B12 levels in individuals with type 2 diabetes.

In Sayedali E et al.(2023)[12] Study showed similar results to our presented work.

In the study by Sayedali et al., the prevailing hypothesis suggests that metformin functions by antagonizing calcium ions, thereby preventing the Ca^{+2}-dependent binding of intrinsic factor (IF)-vitamin B12 complexes to the ileal-cubilin receptor. This interference disrupts the normal ions, thereby preventing the Ca^{+2}-dependent binding of intrinsic factor (IF)-vitamin B12 complexes to the ileal cubilin receptor. This interference disrupts the normal endocytosis process of vitamin B12. Furthermore, it was proposed that metformin may alter the surface charge of the cubilin receptor membrane, rendering it positively charged. This change in charge could repel divalent calcium cations, further compromising. The binding of IF-vitamin B12 complexes to the receptor.[13,14]

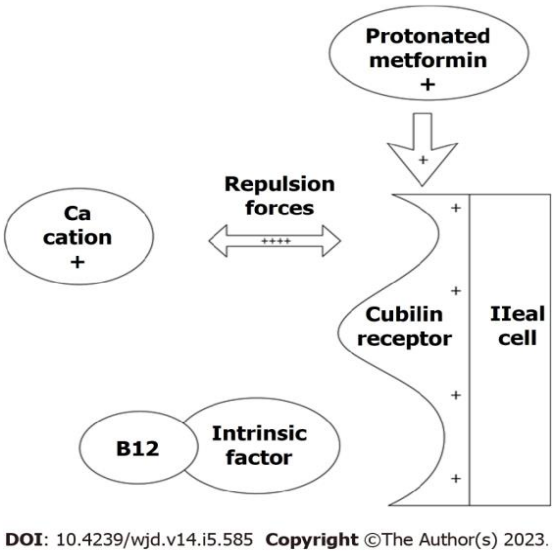

Figure-4: The proposed mechanism through which metformin influences vitamin B12 absorption

In our study, we observed a negative correlation between S. vitamin B12 Levels and both the duration and daily dose of metformin therapy. This finding aligns with the results of Yasitha Kakarlapudi et al.'s (2022) meta-analysis.[15] which reported a higher incidence of vitamin B12 deficiency among metformin users (23.16%) compared to non-users (17.4%). These results highlight the significant impact of metformin dosage and duration on exacerbating B12 deficiency in patients with type 2 diabetes mellitus.

Ethical considerations

The study was approved by the institutional Ethics Committee of Shantabaa Medical College & General Hospital, Amreli.

Limitations

This study had a few limitations. Firstly, the smaller sample size, due to the study short duration, may affect the generalizability of the results, although they may still apply to similar populations in comparable settings. Logistical difficulties prevented us from performing nerve conduction investigations and neuropathy screening. Additionally, we defined vitamin B12 deficiency solely by serum levels, without measuring metabolites like methylmalonic acid and total homocysteine, which are more sensitive indicators.

The cross-sectional design limits our ability to examine causal relationships and risk estimates. Future analytical studies, such as case-control or cohort studies, are recommended to address this limitation and further explore the incidence of B12 deficiency and neuropathy in this population.

CONCLUSIONS

The study identified a high occurrence of vitamin B12 deficiency among the sample population. It also highlighted significant gaps in the recognition and treatment of this deficiency, as it is often undiagnosed. The findings suggest that longer duration and higher doses of metformin therapy are key risk factors for vitamin B12 insufficiency. This deficiency can exacerbate diabetic neuropathy, indicating the need for monthly vitamin B12 assessment in diabetic patients receiving long-term, high-dose metformin treatment. Preventative or therapeutic vitamin B12 supplementation is recommended to mitigate these risks.

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