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*Research Article*

# Relationship between Vitamin B12 levels and Metformin usage in patients with Type 2 Diabetes Mellitus.

**R.Revathi[1*], Prithiv Kumar[2], Rajini Samuel[3], Balaji Rajagopalan[4]**

[1]MSc, Ph.D ,Associate Professor of Biochemistry, Shri Sathya Sai Medical College and Research institute, Sri Balaji Vidyapeeth Deemed to be University, Guduvancherry-thiruporur main road, Ammapettai, Chengalpet district: 603108
[2] II MBBS Student,Shri Sathya Sai Medical College and Research Institute, Sri Balaji Vidyapeeth Deemed to be University
[3]M.D, Professor of Biochemistry, Shri Sathya Sai Medical College and Research Institute, Sri Balaji Vidyapeeth Deemed to be University
[4]M.D, Professor & HOD of Biochemistry, Shri Sathya Sai Medical College and Research Institute, Sri Balaji Vidyapeeth Deemed to be University

***Corresponding author:** DR.R.Revathi
MSc, Ph.D , Department of Biochemistry, Shri Sathya Sai Medical college and Research Institute, Shri Sai Nagar , Sri BalajiVidyapeeth University, Chennai,Ammapetai.
E-mail address: revathimail87@gmail.com

**ABSTRACT:**
Diabetes Mellitus is a chronic, metabolic disease characterized by increased blood glucose levels, which leads to damage to the heart, vasculature, eyes, kidneys and nerves.Over 90% of diabetes mellitus cases were found to be type 2 diabetes mellitus.Vitamin B12 have a role in the etiology of glucose intolerance, and as glucose tolerance worsens, vitamin B12 levels typically fall. Metformin is the most commonly used drug to increase insulin sensitivity in insulin resistant (IR) conditions such as diabetes, prediabetes, polycystic ovary syndrome, and obesity. Several researchers have made recommendations to screen for serum B12 levels in type 2 diabetes mellitus patients treated with metformin .In our study serum Vitamin B12 levels were estimated in Type2 diabetes mellitus patients taking metformin and also compared the serum Vitamin B12 levels in diabetic patients at different age groups includes140 diabetic patients.The Normal serum Vitamin B12 concentration was found to be 14.3% in both male and females with age less than 60 years of age and 10.7% in age group more than 60 years of age. High Serum Vitamin B12 concentration was found to be 42.1% in both male and females with age group less than 60 years of age and 32.9% in age group more than 60 years of age.One way ANOVA WAY Statistical Analysis was done for Serum Vit B12 levels in patients with age less than 60 years and shown in table 5.The *f*-ratio value is 20.20024. The *p*-value is < .00001. The result is significant at $p < .05$. One way ANOVA WAY Statistical Analysis was done for Serum Vit B12 levels in patients with age more than 60 years . The *f*-ratio value is 13.08544. The *p*-value is < .00001. The result is significant at $p < .05$. Our study observed an elevated serum Vitamin b12 levels in diabetic patients may be an indicator of glycemic variability and wider fluctuation in blood glucose level.So monitoring these parameters in diabetic patients may help in predicting and preventing the complications of Diabetes Mellitus.

**KEYWORDS:** Diabetes Mellitus,Vitamin B12, Metformin,Insulin resistant,glucose tolerance,Glycemic variability

***Author for correspondence: Email:** revathimail87@gmail.com*

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## INTRODUCTION

Diabetes mellitus is a collection of metabolic diseases characterized by elevated blood sugar levels brought on by varying degrees of insulin resistance and decreased insulin secretion, ultimately resulting in a relatively low insulin- producing state. The prevalent chronic illness known as type 2 diabetes mellitus (T2DM) is linked to a number of complications that have an major impact on patients quality of life and place a heavy financial and medical burden on society[1.]According to various study reports, vitamin B12 have a role in the etiology of glucose intolerance, and as glucose tolerance worsens, vitamin B12 levels typically fall.[2]

Metformin is the most widely used anti-diabetic drug among type 2 diabetes mellitus patients[1,2].However, metformin induces vitamin B12 malabsorption which may increase the risk of vitamin B12 deficiency among Type2 diabetes mellitus. Metformin is a preferred hypoglycemic drug used for the treatment in type-II diabetic patients. There exist various side effects of metformin like lactic acidosis, abdominal distress and diarrhea. Metformin reduces insulin resistance and fasting plasma insulin level and hence it is called as Insulin Sensitizer. [3,4]

In humans, Vitamin B12 is essential for remethylation of homocysteine to methionine and thereby B12 deficiency could lead to hyperhomocysteinemia, which has been associated with macrovascular complications in people with Type 2 diabetes mellitus.[2] Vitamin B12 deficiency may also leads to peripheral neuropathy in Type 2 diabetes mellitus.[5,6] In addition to that, use of metformin has also been associated with serum vitamin B12 deficiency in various studies. Prolonged use of metformin causes vitamin B12deficiency due to poor absorption by interfering with calcium dependent channels in the ileum.[5,7,8]Certain studies have reported that patients with diabetes have higher serum vitamin B12 levels than those with normal glycemic control, and thereafter fructosamine levels were significantly correlated with serum vitamin B12 levels in patients with DM.[9] The universal routine screening of serum B12 levels among metformin users in type 2 diabetes mellitus is lacking.The purpose of our study is to estimate serum vitamin B12 levels in type 2 diabetes mellitus patients with exposure of metformin.

## MATERIALS AND METHODS

### MATERIALS

This study was approved by ICMR STS project and the result was declared in the month of august.The (Reference ID:2023-10007) and graded as very good project.

**Study Design:** This study will be a cross-sectional study conducted in our institute, Department of Biochemistry.

**Study Period:** Two months from September-October 2023.

**Sample size:** Sample size is calculated with 80% power of test,5% level of significance. The total sample size is 140 subjects including 10% non-response error.

**Inclusion Criteria:**

This study includes 140 type 2 diabetes mellitus patients in the age group between 30-75 yrs. Diabetes mellitus with ≥ one year of diagnosis who are on metformin for at least 6 preceding months were included in the study.

**Exclusion Criteria:**

1. Patients with gastric or intestinal surgeries
2. Patients with malabsorption syndromes
3. Patients with Vitamin B12 supplementation
4. Patients on proton pump inhibitors
5. Patients with pernicious anemia
6. Patient having any critical illness, liver cirrhosis, renal impairment, malignancy, hypothyroidism.

### METHODS

**Estimation of Biochemical parameters:**

Serum Vitamin B12 levels were measured by using Enzyme Linked Immuno Sorbent Assay (ELISA). The normal reference level of Serum Vitamin B12 in adult with age less than 60 years is 200 to 835 pg/ml and with age more than 60 years is 110 to 800 pg/ml.

**Anthropometric measurements:**

Body mass index (BMI) will be used for the assessment of fat distribution and obesity. Using standard measures of height and weight, BMI will be measured using Quetelet's index(BMI=weight(kg)/height(m2).[12] The different groups based on BMI (Body Mass Index) values were mentioned below.

1st group: underweight (BMI= <18.5 Kg/m$^2$),
2nd group: normal weight (BMI = 18.5-24.9 Kg/m$^2$),
3rd group: over weight (BMI = 25-29.9 Kg/m$^2$),
4th group: obese (BMI= 30-34.9 Kg/m$^2$) and
5th group: morbidly obese (>35 Kg/m$^2$)

### RESULT:

The BMI was calculated for all the 140 diabetic patients. Underweight (BMI=<18.5 Kg/m$^2$) and morbidly obese (>35 Kg/m$^2$) patient groups were not found in our sample.

Only normal weight (BMI = 18.5-24.9 Kg/m$^2$), over weight (BMI =25-29.9 Kg/m$^2$) and obese (BMI= 30-34.9 Kg/m$^2$) diabetic patients were found .

In our study Type 2 Diabetic Patients were divided into various groups (1a,1b,2a,2b,3a,3b,4a,4b) based on their age (<60 yrs and > 60yrs ),sex (males and females)and their serum vitamin b12 (low,normal and high values). These are clearly depicted in Table 1. The comparison of BMI in different groups such as normal weight (BMI = 18.5-24.9 Kg/m$^2$), over weight (BMI = 25-29.9

Kg/m$^2$) and obese patients (BMI= 30-34.9 Kg/m$^2$) among the 140 diabetic patients is shown in Table 2.

One way ANOVA WAY Statistical Analysis was done for BMI for all 4 groups with age less than 60 yrs and shown in table 3.The *f*-ratio value is 0.27387. The *p*-value is .844066. The result is *not* significant at $p < .05$.

One way ANOVA WAY Statistical Analysis was done for BMI for 4 groups with age more than 60 yrs and shown in table 4.The *f*-ratio value is 0.33292. The *p*-value is .801559. The result is *not* significant at $p < .05$.

One way ANOVA WAY Statistical Analysis was done for Serum Vit B12 levels in patients with age less than 60 years and shown in table 5.The *f*-ratio value is 20.20024. The *p*-value is < .00001. The result is significant at $p < .05$. One way ANOVA WAY Statistical Analysis was done for Serum Vit B12 levels in patients with age more than 60 years and shown in table 6. The *f*-ratio value is 13.08544. The *p*-value is < .00001. The result is significant at $p < .05$.

Comparison of Serum vitamin B12 Levels in different Age groups of the 140 diabetic patients were shown in the figure 1. The Normal serum Vitamin B12 concentration was found to be 14.3% in both male and females with age less than 60 years of age and 10.7% in age group more than 60 years of age. High Serum Vitamin B12 concentration was found to be 42.1% in both male and females with age group less than 60 years of age and 32.9% in age group more than 60 years of age.

**TABLE 1: Serum Vitamin B12Levels and BMI seen in different groups of 140 diabetic patients**

| **Groups** | **Group details** | **BMI (kg/m$^2$) Mean± S.D** | **Vit B12(pg/ml)** |
|---|---|---|---|
| 1a | Females<60yrs with Normal B12 | 24.69 ± 3.73 | 626.95 ± 159.87 |
| 1b | Males <60 yrs with Normal B12 | 23.85 ± 1.52 | 638.08 ± 241.11 |
| 2a | Females <60 yrs with High B12 | 24.14 ± 3.01 | 1111.40 ± 322.14 |
| 2b | Males <60 yrs with High B12 | 25.00 ± 4.09 | 1395.54 ± 429.96 |
| 3a | Females>60yrs with Normal B12 | 23.31 ± 1.73 | 596.25 ± 170.21 |
| 3b | Males >60 yrs with Normal B12 | 25.19 ± 5.05 | 562.90± 172.48 |
| 4a | Females >60 yrs with High B12 | 24.09 ± 2.24 | 1166.93 ± 452.52 |
| 4b | Males >60 yrs with High B12 | 24.12 ±4.16 | 1354.48 ±420.26 |

**Table2: Comparison of BMI seen in differentgroups of 140 diabetic patients**

| **Groups** | **Group details** | **Total no of normal weight individuals (BMI:18.5-24.9 Kg/m$^2$)** | **Total no of over weight (BMI: 25-29.9 Kg/m$^2$)** | **obese (BMI: 30-34.9 Kg/m$^2$)** | **Total** |
|---|---|---|---|---|---|
| 1a | Females<60yrs with Normal B12 | 10 | 04 | 01 | 15 |
| 1b | Males <60 yrs with Normal B12 | 04 | 01 | 0 | 05 |
| 2a | Females <60 yrs with High B12 | 12 | 0 | 01 | 13 |
| 2b | Males <60 yrs with High B12 | 30 | 10 | 06 | 46 |
| 3a | Females>60yrs with Normal B12 | 09 | 01 | 0 | 10 |
| 3b | Males >60 yrs with Normal B12 | 04 | 0 | 01 | 5 |
| 4a | Females >60 yrs with High B12 | 13 | 04 | 0 | 17 |
| 4b | Males >60 yrs with High B12 | 18 | 08 | 03 | 29 |

**TABLE 3 BMI for 4 groups less than 60 yrs one way ANOVA WAY Statistical Analysis:**

| paramete r | Normal Serum Vit B12 Females <60 yrs | Normal Serum Vit B12 Males <60yrs | High Serum Vit B12 Females <60yrs | High Serum Vit B12 Males <60yrs | Total |
|---|---|---|---|---|---|
| N | 15 | 5 | 13 | 46 | 79 |
| ∑X | 370.34 | 119.25 | 313.8 | 1149.78 | 1953.17 |
| Mean | 24.6893 | 23.85 | 24.1385 | 24.9952 | 24.724 |
| $\sum X^2$ | 9338.7242 | 2853.3163 | 7683.6182 | 29491.594 | 49367.25 27 |
| Std.Dev. | 3.7347 | 1.5169 | 3.0134 | 4.0895 | 3.7171 |

The *f*-ratio value is 0.27387. The *p*-value is .844066. The result is *not* significant at $p < .05$.

**TABLE 4:one way ANOVA WAY Statistical Analysis for BMI for 4 groups with patients age more than 60 yrs**

| parameter | Normal Serum B12 F>60yrs | Normal Serum B12 M>60yrs | High Serum B12 F >60yrs | High Serum B12 M>60yrs | Total |
|---|---|---|---|---|---|
| N | 10 | 5 | 17 | 29 | 61 |
| ∑X | 233.09 | 125.96 | 409.54 | 699.5 | 1468.09 |
| Mean | 23.309 | 25.192 | 24.0906 | 24.1207 | 24.067 |
| $\sum X^2$ | 5460.0695 | 3275.0448 | 9946.443 | 17357.5646 | 36039.1219 |
| Std.Dev. | 1.7312 | 5.0463 | 2.2414 | 4.1625 | 3.4315 |
| **The *f*-ratio value is 0.33292. The *p*-value is .801559. The result is *not* significant at $p < .05$.** | | | | | |

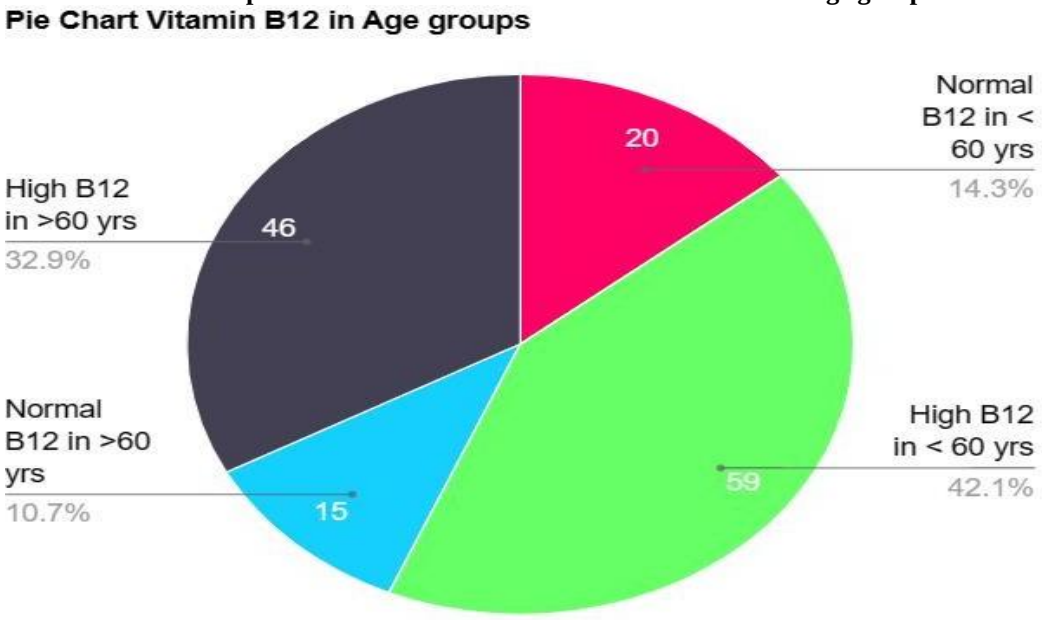
**TABLE 5:One way ANOVA Statistical analysis for Serum Vit B12 with patients age less than 60 years**

| parame ter | Normal Serum B12 F <60yrs | Normal Serum B12 M<60yrs | High Serum B12 F <60yrs | High Serum B12 M<60yrs | Total |
|---|---|---|---|---|---|
| N | 15 | 5 | 13 | 46 | 79 |
| ∑X | 9404.245 | 3190.391 | 14448.158 | 64194.998 | 91237.792 |
| Mean | 626.9497 | 638.0782 | 1111.3968 | 1395.5434 | 1154.909 |
| $\sum X^2$ | 6253807.477 | 2268245.712 | 17302936.2516 | 97905751.0635 | 123730740.5833 |
| Std.De v. | 159.8703 | 241.1052 | 322.1413 | 429.9572 | 485.1568 |
| The *f*-ratio value is 20.20024. The *p*-value is < .00001. The result is significant at $p < .05$. | | | | | |

**TABLE 6:one way ANOVA Statistical analysis for Serum Vit B12 with patients age more than 60 years**

| paramete r | Normal Serum B12 F>60yrs | Normal SerumB12 M>60yrs | High SerumB12 F >60yrs | High SerumB12 M>60yrs | Total |
|---|---|---|---|---|---|
| N | 10 | 5 | 17 | 29 | 61 |
| ∑X | 5962.459 | 2814.501 | 19837.797 | 39279.846 | 67894.603 |
| Mean | 596.2459 | 562.9002 | 1166.9292 | 1354.4774 | 1113.026 |
| $\sum X^2$ | 3815835.2153 | 1703287.1295 | 26425680.0188 | 58148970.9026 | 90093773.2663 |
| Std.Dev. | 170.2102 | 172.4847 | 452.519 | 420.2595 | 492.0247 |
| The *f*-ratio value is 13.08544. The *p*-value is < .00001. The result is significant at $p < .05$. | | | | | |

**FIG 1:Comparison of Serum vitamin B12 Levels in Different Age groups**




**DISCUSSION**

Vitamin B12 are involved in the pathogenesis of glucose intolerance. The levels of vitamin B12 tend to decrease with increasing severity of glucose tolerance. It is well known that vitamin B12 deficiency is associated with increased risk of gestational diabetes mellitus. A recent study also reported that supplementation with vitamin B12 can improve glycemic control and insulin resistance in type 2 diabetic patients. Previous studies had found that free vitamin B12 can be absorbed passively without utilizing the intrinsic factor. Therefore,exogenous vitamin B12 supplementation may leads to absorption of vitamin B12 via a passive pathway and this can increase serum B12 levels. However, long-term vitamin B12 administration can trigger production of anti-TCB II antibodies and reduce TCB II clearance. This can impairs intracellular utilization of vitamin B12 and can aggravate the intracellular vitamin B12 deficiency. Serum vitamin B12 levels in peripheral circulation will be high but intracellular deficiency will be seen that may may affect cellular glucose metabolism and homeostasis in diabetic patients.[8,9,10]

The diabetic patients had significantly higher total B12 binding capacity, higher serum B12 levels and unsaturated B12 binding capacity when compared with the normal controls and the fructosamine levels were correlated significantly with the change of total B12 binding capacity and serum B12 levels in diabetic patients. These results indicate the effects of glycemic control on B12 metabolism in diabetes mellitus.The correlation between serum vitamin B12 levels and glycemic fluctuation in patients with type 2 diabetes mellitus was reported. Therefore the serum vitamin B12 may be a potentially useful indicator of glycemic fluctuation in diabetic patients, regardless of metformin therapy.[13,14]

The mean amplitude of glycemic excursions helps to characterize the diabetic instability that denotes the fluctuations in blood glucose levels.The measurement of amplitude of glycemic excursion was small for normals, larger for stable diabetics, and largest for unstable diabetics.[15] Moreover, evaluation of daily glucose fluctuations provides baseline information for the clinical management of T2DM. Therefore, regular glycemic fluctuation monitoring should be considered an important component of routine clinical management of patients with T2DM [15,16,17]any studies have shown decreased vitamin B12 values in diabetic patients treated with metformin. But in our study serum vitamin B12 values were not lowered in any of the 140 diabetic patient samples. The serum B12 values were found to be normal in 35 and increased in 105 diabetic patients. The diseased conditions like solid neoplasms, haematologic malignancies, renal failure and liver diseases were not included in our study. The Previously published research articles emphasized that high B12 values seen in diabetic patients treated with metformin are due to Glycemic variability. The fluctuation in blood glucose causes elevated b12 levels in this patients. In our study the results obtained are similar and so the high B12 values observed in our diabetic patients may be due to glycemic variability and glucose fluctuations.The glycemic variability and fluctuations in blood glucose levelwere estimated. This is the limitation of our study.The future scope of our study is to estimate and correlate the glycemic variability and serum vitamin b12 in diabetic patients treated with metformin.

**CONCLUSION**

Elevated serum Vitamin B12 levels in diabetic patients may be an indicator of glycemic variability and wider fluctuation in blood glucose level.So monitoring these parameters in diabetic patients may help in predicting and preventing the complications of Diabetes mellitus.

**CONFLICT OF INTEREST:**

NIL

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