



**PREVALENCE OF LOW VITAMIN B12 LEVELS IN PATIENTS WITH TYPE 2  
DIABETES MELLITUS ON METFORMIN IN A TERTIARY TEACHING CARE  
HOSPITAL**

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**ABSTRACT**

**Introduction:** Metformin is a biguanide and a commonly used oral hypoglycemic agent in treatment of type 2 diabetes mellitus. Duration of diabetes significantly affects the outcome in both treated and untreated patients. It is a known fact that age, duration of diabetes can affect the vitamin B12 status of a patient. Low vitamin B12 level is said to occur in diabetics due to metformin therapy also. There is no universal recommendation to supplement vitamin B12, especially in high risk populations. Studying the biochemical profile to detect vitamin B12 deficiency in these populations will provide useful data to support the need for supplementation. **Objectives:** To assess the biochemical level of Vitamin B12 in metformin treated type 2 diabetic patients and also to assess the relation between duration of diabetes & development of vitamin B12 deficiency. **Methodology:** This is a cross sectional observational study of serum vitamin B12 concentration in patients taking metformin for management of type 2 diabetes in GMKMC, Salem. All patients on metformin attending the diabetes outpatient clinics in past six months has been taken up for study after getting informed consent from them. Those on current B12 treatment for any condition were excluded. Data collected included age, duration of disease and metformin therapy, vitamin B12 concentration. Patients were grouped into 3 categories based on their of serum vitamin B12 concentration: **Results:** Duration of disease and duration of metformin therapy has positive correlation with presence of vitamin b12 deficiency which is also statistically significant with P value less than 0.05, whereas deficiency is much more common in old age patients more than 60 years. **Conclusion:** Metformin therapy is associated with a higher prevalence of biochemical B<sub>12</sub> deficiency. Both duration of disease and drug has positive impact on Vitamin B12 level. Hence screening of B12 is advisable in chronic diabetics.

**KEYWORDS:** Diabetes mellitus, Metformin, Vitamin B12 deficiency.

**INTRODUCTION**

Metformin is an important drug in management of diabetes. American Diabetic Association recommends it as a first-line treatment for the prevention of type 2 diabetes in individuals with pre-diabetes.<sup>[1]</sup> Metformin increases insulin sensitivity in the liver and decreases glucose production. It is also used to treat polycystic ovarian syndrome, a disease linked to high blood glucose levels.<sup>[2]</sup>

Vitamin B<sub>12</sub>, commonly called as cobalamin, belongs to group of water-soluble vitamin mainly required for hematopoietic and neurological functions in the body. It is mainly rich in foods from animal sources such as meat and fish. When vitamin B<sub>12</sub> from animal foods enters the stomach it is protein bound and must be released by the action of pepsin in the stomach. It is then attached to R-proteins and is transported through the duodenum where

it is hydrolyzed and free cobalamin is released.<sup>[3]</sup> It requires intrinsic factor (IF) in order to be absorbed in the terminal ileum. This absorption process is predominantly calcium mediated.

Vitamin B<sub>12</sub> is an essential cofactor in the conversion of homocysteine to methionine and regeneration of folate. This step is has utmost importance in the metabolic process that leads to DNA synthesis and formation and protection of the myelin sheath. Demyelination of nerve endings can lead to peripheral neuropathy. Hence B12 deficiency symptoms begin to manifest as numbness and paraesthesia in the feet. This tingling feeling is caused by pressure on the nerves. If the vitamin B<sub>12</sub> deficiency is not corrected, weakness, ataxia, sphincter disturbance, and changes in mental status will follow.<sup>[4]</sup>

It was in 1971 researchers began to speculate that one of important adverse effects of metformin use was vitamin B<sub>12</sub> malabsorption.<sup>[5]</sup> Current research points to the effect of metformin on the calcium dependent B<sub>12</sub>-intrinsic factor complex and absorption in the terminal ileum as the primary mechanism for vitamin B<sub>12</sub> depletion.<sup>[6]</sup> Although a substantial amount of research has been conducted during the past forty-five years regarding metformin and vitamin B<sub>12</sub> deficiency, screening and assessment has never been included as part of the drug protocol. Recently, two large human clinical trials have released findings, further strengthening the evidence linking metformin use and vitamin B<sub>12</sub> depletion and thus a possible need for routine vitamin B<sub>12</sub> assessment in patients using metformin.<sup>[7,8]</sup> The prevalence of low serum B12 concentration in diabetics using metformin in our Salem area is unknown. The aim of this study was to estimate the prevalence of low serum B12 in those taking metformin and also to analyse the relationship between duration of treatment on severity of deficiency.

## METHODS

This is a cross sectional observational study of serum vitamin B12 concentration in patients taking metformin for management of type 2 diabetes in GMKMC, Salem. All patients on metformin attending the diabetes outpatient clinics at Government Mohan Kumaramangalam medical college hospital, Salem in past six months has been taken up for study after getting informed consent from them. Those on current B12 treatment for any condition were excluded.

Data collected included age, duration of disease and metformin therapy, vitamin B12 concentration. Patients were grouped into 3 categories based on their of serum vitamin B12 concentration: Vitamin B12 deficiency (<150 pmol/L), borderline vitamin B12 (150–219

pmol/L) and normal vitamin B12 ( $\geq 220$  pmol/L). Data were analysed using SPSS version 21.0. The primary outcome results are presented as arithmetic means. Assessment for significance between means was tested using ANOVA. All tests were two-sided and p values of <0.05 were considered to be statistically significant. Ethics approval for the study was obtained from Institutional ethics committee.

## RESULTS

### Demographics

A total of 50 patients were selected for study who satisfied our inclusion criteria. Among them the sex distribution was almost similar with 24 males and 26 females in the study. Most of the patients in our study were above 60 year of age with around 35 patients above 60 years. Among our study group 37 patients (74%) were on metformin for more than ten years will rest were on metformin for less than 10 years. We also evaluated the duration of diabetes in these patients and around 56 % of patients was having diabetes more than ten years and the rest having less than 10 years.

### Vitamin B12 concentration

The distribution of serum vitamin B12 concentration was as expected to previous studies where the number of patients with deficiency was around 12 which is around 24% while thirty patients (60%) had borderline levels while the rest has normal levels. We also analysed the peripheral smear picture in these patients and while majority of patients had normal picture around 24% (N=12) of patients had macrocytosis which is a prominent picture in B<sub>12</sub> deficiency. Where as microcytosis was seen in six patients all were having borderline B<sub>12</sub> levels. Macrocytosis is predominantly seen in the deficient group. Other groups also have macrocytosis, but in lower number.

| SMEAR        | VIT B12 LEVEL |            |        |
|--------------|---------------|------------|--------|
|              | DEFICIENT     | BORDERLINE | NORMAL |
| MACROCYTOSIS | 7             | 2          | 3      |
| MICROCYTIC   | 0             | 6          | 0      |
| NORMAL       | 5             | 22         | 5      |

There was no impact of gender on Vitamin B12 concentrations as there was not much difference in number of patient's deficient between both groups. Whereas B12 levels reduced with increasing age which was statistically significant (P=0.024), Where patients more than 60 years of age has low B12 levels compared to below 60 patients. Hence older age predicted lowered serum Vitamin B12 status (<220 pmol/L).

Finally we analysed the effect of duration of diabetes and duration of metformin therapy on the vitamin B12 levels. First we analysed the duration of metformin on vitamin B12 levels. We grouped the patients into those who are taking metformin for more than ten years and less than 10 years and there was significant difference in deficiency status between the patients with P value of 0.041. Where increase in number years of treatment with metformin ends up in more deficiency.

| VITAMIN B12 LEVELS | DURATION OF METFORMIN |          |
|--------------------|-----------------------|----------|
|                    | > 10 YRS              | < 10 YRS |
| DEFICIENT          | 4                     | 8        |
| BORDERLINE         | 7                     | 23       |
| NORMAL             | 2                     | 6        |

Similarly duration of diabetes also has an positive correlation on vitamin B12 deficiency where the duration of disease if more than ten years has an higher incidence

of deficiency compared to patients who are diabetic less than ten years which is also statistically significant with P value of 0.038.

| VITAMIN B12 LEVELS | DURATION OF DM |          |
|--------------------|----------------|----------|
|                    | > 10 YRS       | < 10 YRS |
| DEFICIENT          | 6              | 6        |
| BORDERLINE         | 14             | 16       |
| NORMAL             | 2              | 6        |

## DISCUSSION

The American Diabetic association recommends metformin as the first line of treatment for glycaemic control in type 2 diabetes mellitus once lifestyle modifications is not enough.<sup>[9]</sup> Although it is advisable to do annual screening of serum B12 concentration in patients with type 2 diabetes on metformin for longer periods it is not followed due to financial and practical reasons.

The overall level of prevalence of reduced serum B12 concentration (<220 pmol/L) in patients with type 2 diabetes receiving metformin therapy in this study was 24% which is similar to results from other studies reporting between 5 and 36.8%.<sup>[10]</sup> Where one study done in North America by Reinstatler et al reported a vitamin B12 deficiency in only 5.8% of patients with type 2 diabetes taking metformin. This is in contrast to a study in another study by Nervo et al there was high prevalence of decreased serum B12 of 36.8%. Differences between the two studies may be due to lot of factors like ethnicity, vitamin supplementation etc.

The 2003–2006 American National Health and Nutrition Examination Survey (NHANES), reports the mean population serum Vitamin B12 level was 370 pmol/L compared with 296 pmol/L in the present study.<sup>[11]</sup> Again this may reflect the impact of geographical location of the patients.

There are few data on Vitamin B12 status in the general population in our state based on age whereas few studies done on this aspect has mentioned the average level to be 245 pmol/L, in people with average age of 40.<sup>[12]</sup> In an previous study older age group (age >65 years) 35.5% were observed to have a lowered serum Vitamin B12 concentration (<221 pmol/L).<sup>[13]</sup> This compares with a rate of 26% in patients >60 years in the current study, which reflects the influence of age alone on B12 status.

The P value for duration of diabetes is calculated by dividing the group into two subgroups. There were more people below 10 years for duration of treatment with metformin. Here the patients who were on metformin for more than ten years had increased deficiency of metformin show significance influence which could be due to prolonged effect of the drug on the metabolism of B<sub>12</sub>. The dosage of the metformin was not calculated. So the significance of duration in this small group may be

affected by total dose of metformin. Also the mean duration of therapy with metformin is high in the deficient group followed by the borderline group. So seeing this we can say that when the duration of therapy with metformin there is increasing chance for development of vitamin deficiency. The same can be said on the duration of diabetes too. The National health & Nutritional survey done from US 1999-2006 had documented vitaminB<sub>12</sub> deficiency in both diabetics with and without metformin therapy. But the biochemical B12 deficiency is higher in group with metformin. It is correlated with our study where we found some relation with mean duration of metformin and B12 deficiency.

As this is a cross-sectional study and we do not have B12 concentrations prior to commencement of metformin, it cannot say outrightly that metformin is causative of low B12 concentrations. Furthermore, the clinical implications of this are less clear and whether supplementation of these individuals would be of benefit requires an intervention study.

While it is difficult to diagnose B12 deficiency based on physiological symptoms, further data on PCV and presence of megaloblastic anaemia would also be useful. This would help to conclude whether universal screening of Vitamin B12 for those taking metformin is appropriate. Given the low rate of reduced B12 levels in those less than 60 years, it may be argued that screening could be safely restricted to those older than 60 years.

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