

Research Article

A comparative study of levels of vitamin B12 in patients of type 2 diabetes mellitus on metformin and not on metformin at tertiary care center

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ABSTRACT

Background: Type 2 diabetes mellitus is associated with vitamin B12 deficiency which is further increased in patients who are on metformin therapy. The objective of this study is to determine the frequency of vitamin B12 deficiency among Indian patients with type 2 diabetes mellitus on metformin therapy compared to those who are not on metformin.

Methods: The study was a hospital based, case control type of analytical study conducted in SMS Medical College & Hospital, Jaipur during a period of 1 year. The study group has 50 patients with a diagnosis of T2DM and a prescription history of metformin for ≥ 18 months and control group has 50 patients with diagnosis of T2DM and no history of metformin use in the past five years. The following data was recorded for each patient: age, sex, weight, height, body mass index (BMI), years with diabetes, total daily dose of and years on metformin. Serum vitamin B12 was measured using an immunoassay method. Data were statistically analyzed.

Results: Mean serum vitamin B12 levels in the study group was 431.84 ± 265.76 and in control was 744.76 ± 271.927 and the difference was statistically significant. Mean serum vitamin B12 levels in vegetarians (547.27 ± 303.011) were significantly lower than in non-vegetarian (699.22 ± 307.992) (p value 0.029). A significant negative correlation existed between the S. vitamin B12 and duration of diabetes ($r = -0.445$) by using Pearson's correlation coefficient in study group.

Conclusions: Metformin is significantly associated with decrease in vitamin B12. So we recommend that vitamin B12 should be measured prior to initiation of metformin therapy and later annually in patients of type 2 diabetes mellitus who are on metformin therapy for long duration ($\geq 3-4$ years) or in higher doses (≥ 2 gm/day).

Keywords: Vitamin B12, Type 2 diabetes mellitus, Metformin

INTRODUCTION

Diabetes mellitus (DM) type 2 is a heterogeneous group of disorders characterized by variable degree of insulin resistance, impaired insulin secretion and increased glucose production. Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the

disease.^{1,2} With an ever-increasing incidence of type 2 diabetes in the Indian population, continual assessment must be made to ensure the ongoing evaluation of treatment options and to ensure patient care is not compromised.

Metformin, a biguanide, is the first-line oral hypoglycaemic agent used in the treatment of the

overweight or obese patient with T2DM. Metformin acts on several tissues via activation of the adenosine monophosphate-activated protein kinase (AMPK) system to reduce serum glucose. The primary effect of metformin is to suppress hepatic gluconeogenesis.³ Side effects of metformin therapy are gastrointestinal upset and rarely lactic acidosis. Another clinically relevant side effect of metformin therapy is vitamin B12 deficiency. This side effect is well described, frequently forgotten and not routinely screened for. In 2009, Pflipsen et al showed that 22% of patients with T2DM had a vitamin B12 deficiency, and those on metformin had reduced serum vitamin B12 levels with an increased risk of vitamin B12 deficiency.⁴ The mechanism by which metformin therapy causes vitamin B12 deficiency is not clear, but it is thought to be due to either alterations in small bowel motility, which stimulate small bowel bacterial overgrowth and subsequent vitamin B12 deficiency, or by directly decreasing vitamin B12 absorption.^{2,5}

The frequency of vitamin B12 deficiency in patients with T2DM on metformin therapy in India is unknown and the measurement of serum vitamin B12 in T2DM patients on metformin is not part of the standard annual review examination. The objective of this study is to determine the frequency of vitamin B12 deficiency among Indian patients with T2DM on metformin therapy compared to those who are not on metformin.

METHODS

The study was a hospital based, case control type of analytical study conducted in SMS Medical College & Hospital, Jaipur during a period of 1 year. Sample size was calculated as per the reference article⁶ the expected proportion of vitamin B12 deficiency in type 2 diabetes patients on metformin is 33%. And those not on metformin is 7.5%. The sample size required to verify difference in these proportion at 95% confidence and 80 power will be 46 cases in each group. As a whole number 50 cases were taken in each group. Therefore study group has 50 patients with a diagnosis of T2DM and a

prescription history of metformin for ≥ 18 months and control group has 50 patients with diagnosis of T2DM and no history of metformin use in the past five years.

Patients with diagnosis of pernicious anaemia, vitamin B12 supplementation, malabsorption (celiac disease, inflammatory bowel disease, gastrointestinal surgery), malnutrition (pure vegans, anorexia nervosa), iron deficiency anaemia, history of thyroid disease and thyroxin treatment and/or a history of other organ-specific autoimmune conditions (vitiligo, Addison's, primary ovarian failure, hypoparathyroidism) were excluded.

The following data was recorded for each patient: age, sex, weight, height, body mass index (BMI), years with diabetes, total daily dose of and years on metformin. The date of the earliest documentation of a diagnosis of diabetes was used to calculate the number of years with diabetes. The number of years on metformin was calculated based on the date of the earliest medical record documenting the use of metformin. The dose of metformin was based on the most recent medical record documenting the dose.

Serum vitamin B12 was measured using an immunoassay with a co-efficient of variation of approximately 10% or less. Data were analysed using student's t-test (Microsoft Excel 2007) and Fisher's test (GraphPad 2007).

RESULTS

Table 1 shows demographic profile of the patients with type 2 diabetes.

The youngest patient was aged 42 years while the oldest patient was aged 60 years. Mean age of the study group was 51.98 ± 5.17 years, while it was 49.28 ± 5.08 in control group, which was statistically non-significant.

44% patients in study group and 48% patients in controls were male.

Table 1: Demographic profile of the patients with type 2DM

| Variables | Study group (n = 50) | Control group (n = 50) | P value |
|-----------------------|-------------------------|---------------------------|------------------------|
| Mean age (years) | 51.98 \pm 5.17 | 49.28 \pm 5.08 | 0.10 (Non significant) |
| Gender | | | |
| Male | 22 (44%) | 24 (48%) | |
| Female | 28 (56%) | 26 (52%) | |
| Mean BMI | 24.27 \pm 3.73 | 22.61 \pm 2.98 | 0.016 (Significant) |
| Dietary Habits | | | |
| Vegetarian | 36 (72%) | 36 (72%) | |
| Non-vegetarian | 14 (28%) | 14 (28%) | |
| Years with diabetes | 6.15 \pm 3.33 | 3.44 \pm 1.82 | 0.000 (significant) |

In the study group BMI ranged from 17 to 28kg/m² with a mean of 24.27±3.73 kg/m². In the control group BMI ranged from 17 to 30kg/m² with a mean of 22.61±2.98 kg/m². Significantly higher mean was observed in mean BMI in study group as compared to controls (p=0.016). No of vegetarians and non-vegetarians were equal in both the groups.

The duration of diabetes in case ranged from 1.5 to 12 years with the mean of 6.15±3.33 years while in controls it ranged from 1.5 to 10 years with a mean of 3.44±1.82 years. The difference between two groups was statistically significant with p value of 0.000.

The mean RBS in cases was 163.14±38.61 while mean RBS in control was 177.70±28.59. The difference between two is significant (p=0.035). The mean glycated hemoglobin in study group (7.84±1.07) was significantly lower than mean glycated hemoglobin in control group (8.32 ±1.2) with p value of 0.041(Table 2).

Table 2: Distribution of mean RBS and glycated Hb in study and control group.

| | Study group (n = 50) | Control group (n = 50) | P value |
|-------------|-------------------------|---------------------------|------------------------|
| RBS | 163.14±38.61 | 177.70 ±28.59 | 0.035 (significant) |
| Glycated Hb | 7.84 ±1.07 | 8.32±1.22 | 0.041 (significant) |

In the cases group S. vitamin B12 ranged from 155 to 980 pg/ml with a mean of 431.84±265.76. In the control group S. vitamin B12 ranged from 162 to 1268 pg/ml with a mean of 744.76±271.927.

Significantly lower mean S. vitamin B12 was observed in cases as compared to controls (p=0.000) (Table 3).

Table 3: Distribution of mean vitamin B12 in study and control group.

| | Study Group (n = 50) | Control Group (n = 50) | P value |
|-------------------|-------------------------|---------------------------|------------------------|
| Serum vitamin B12 | 431.84± 265.76 | 744.76±271.93 | 0.000 (significant) |

Mean serum vitamin B12 levels in vegetarians (547.27±303.011) were significantly lower than in non vegetarian (699.22±307.992) (p value 0.029).

Table 4: Distribution of vitamin B12 between vegetarian and non-vegetarian.

| Diet | Number | Mean S. Vitamin B12 | P-Value |
|----------------|--------|---------------------|----------------|
| Vegetarian | 72 | 547.27 ± 303.011 | 0.029 |
| Non Vegetarian | 28 | 699.22 ±307.992 | (significant) |

Table 5: Distribution of mean vitamin B12 according to duration of diabetes.

| Duration of diabetes | Study group | | Control group | | P value |
|----------------------|---------------------|------------------------------|-------------------------|------------------------------|---------------------------|
| | No. | Mean vitamin B ₁₂ | No. | Mean vitamin B ₁₂ | |
| ≤ 5 years | 30 | 505.47±275.24 | 46 | 754.00±265.55 | 0.0002 highly significant |
| >5 years | 20 | 321.40±212.43 | 4 | 638.50±365.59 | 0.024 significant |
| P | 0.015 (significant) | | 0.421 (not significant) | | |

Mean S vitamin B12 in study group among patients with duration of DM of less than 5 years was 505.47±275.24 pg/ml, while mean S. vitamin B12 in patients with duration of diabetes more than 5 years was 321.40 ±212.43 pg/ml. The difference between the two is statistically significant (p = 0.015). Mean S vitamin B12 in control group among patients with duration of DM of less than 5 years was 754.00 ±265.55 pg/ml, while mean S. Vitamin B12 in patients with duration of diabetes more than 5 years was 638.50 ±365.59 pg/ml. The difference between the two is statistically non-significant (p = 0.421). The difference in mean vitamin B12 in both the groups was significantly difference irrespective of duration of diabetes (Table 5).

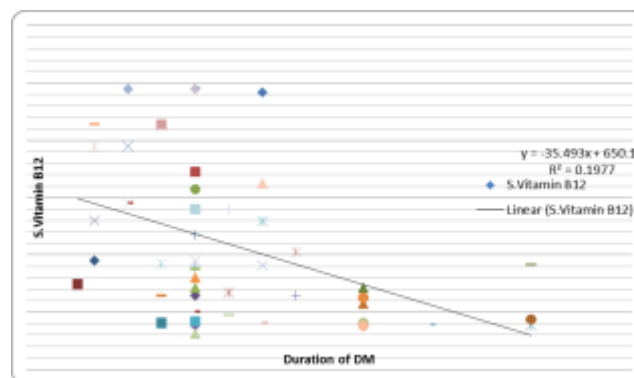


Figure 1: Correlation between duration of diabetes and serum vitamin B12.

A significant negative correlation existed between the s. vitamin B12 and duration of diabetes ($r=-0.445$) by using Pearson's correlation coefficient in study group stating that as duration of diabetes increased the Vitamin B12 levels decreased and vice-versa. $R^2=0.1977$, shows that only 19.77% of the total variation in vitamin B12 levels was explained by linear relation with duration of diabetes (Figure 1).

DISCUSSION

Out of 100 patients studied, 64 were males and 36 were females. Male: female ratio was 1.5:1. The mean age of cases was 51.98 ± 5.17 and the range was 45 to 86. The controls were appropriately age and sex matched with mean age of 49.28 ± 5.08 years. Thus, the mean age and the range of age of controls were same as cases without any statistically significant difference ($p=1.0$). These findings are consistent with the findings of Gale EA, Gillespie KM who observed a male: female ratio of 3:2.⁶ In another study Omar Marar et al 2011 reported a male: female ratio of 1.7:1 and mean age of study group as 64 ± 10.6 years.³

Mean BMI of patients in study group was 24.266 ± 3.72 kg/m^2 . BMI ranged from 17 to 28 kg/m^2 . In the control group BMI ranged from 17 to 30 kg/m^2 with a mean of 22.60 ± 2.9761 kg/m^2 . Significantly higher mean was observed according to mean BMI in cases as compared to controls ($p=0.016$). Similar results were observed by studies done in the past. A large proportion of Asian and Chinese patients with type 2 diabetes are of normal weight: a recently published pooled cross-sectional analysis of 39,794 diabetes patients from Asia (most of whom had type 2 diabetes), revealed that 64% had a BMI $<25 \text{ kg/m}^2$. One regional Chinese study showed that 59.2% of 521 diabetes patients in Hong Kong had a BMI $<25 \text{ kg/m}^2$ and another study showed that 36% of 4,160 patients in Shanghai had a BMI $<24 \text{ kg/m}^2$.^{8,9}

In our study, the duration of Diabetes in cases ranged from 1.5 to 12 years with the mean of 6.1 ± 3.32 years while in controls it ranged from 1.5 to 10 years with a mean of 3.4 ± 1.81 years. The difference between two groups was statistically significant with p value of .000. The mean duration of diabetes was higher in the study done by Josie M M Evans who observed a mean duration as 8.5 ± 6.4 years.¹⁰

The mean s. vitamin B12 in vegetarians was 547.27 pg/ml which was significantly lower than mean s. vitamin B12 seen in non-vegetarian which was 699.22 pg/ml with a p value of 0.029. In a study by Yajnik et al to determine the frequency of vitamin B12 deficiency and hyperhomocysteinemia among 441 healthy middle aged Indian men, vitamin B12 deficiency as defined by vitamin B12 concentrations $<150 \text{ pmol/L}$ was reported among 67% of the study participants.¹¹ Vegetarian diet was the sole significant factor associated with low

vitamin B12 levels in this study on multivariate analysis (OR 4.4 95% CI 2.1-9.3).

It is found in our study that in the cases S. vitamin B12 ranged from 155 to 980 pg/ml with a mean of 431.84 ± 265.76 . In the controls S. vitamin B12 ranged from 162 to 1268 pg/ml with a mean of 744.76 ± 271.927 . Significantly lower mean S. vitamin B12 was observed in cases as compared to controls ($p=0.000$), similar to results from other studies (Mazokopakis & Starakis, 2012,¹² Reinstatler et al.^{12,13} In one early randomised controlled trial by DeFronzo et al, metformin decreased the serum vitamin B12 levels by 22% and 29% compared to placebo and glyburide respectively.¹⁴ Several cross sectional studies, and case reports have documented an increased frequency of vitamin B12 deficiency among type 2 DM (T2DM) patients.^{4,15-19} Metformin use has been unequivocally demonstrated as the prime factor associated with vitamin B12 deficiency among patients with T2DM.²⁰⁻²⁴

It was observed that prevalence of decreased serum vitamin B12 status in metformin treated patients in our study was lower than those in the Green et al study.²⁴ This comparison must be interpreted with caution as there are other factors that may affect the serum Vitamin B12 of these patients which were not addressed in this study (diet, drug interactions etc.). Furthermore as the patients recruited into the Green et al study were non-institutionalized there may have been a level of malnutrition in regards to nutritional intake as has been observed in such populations (McElnay et al).²⁵

The mechanism of metformin inhibition of absorption is not fully understood. The current theory of inhibition is based on interference with intrinsic factor binding in the gut. It would be assumed that an increase in concentration of the inhibiting substance (metformin) would result in an increase in inhibition of said absorption. As metformin is excreted unchanged in urine with an elimination half-life of approximately 5 hours (Graham et al) it would be logical for the relationship between metformin and vitamin B12 malabsorption to be somewhat linear.²⁶

CONCLUSION

From present study it was concluded that metformin which is first line oral hypoglycemic agent as recommended by ADA is significantly associated with decrease in vitamin B12. So we recommend that vitamin B12 should be measured prior to initiation of metformin therapy and later annually in patients of type 2 diabetes mellitus who are on metformin therapy for long duration ($\geq 3-4$ years) or in higher doses ($\geq 2 \text{ gm/day}$).

Measurement of the serum vitamin B12 concentrations should be the preliminary screening step for vitamin B12 deficiency among patients with T2DM.

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Ethical approval: Not required

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