"ASSESSMENT OF VITAMIN B12 STATUS IN TYPE 2 DIABETIC SUBJECTS"

Dr. Kapil Shrivatsava[1] (First Author),
Assistant Professor, G R Medical College, Gwalior, MP

Dr. Sushma BJ(Corresponding author),
Associate Professor, Dept. of Biochemistry, PIMS, Lucknow, UP
sushmabj1983@gmail.com

ABSTRACT

Background: The increasing incidence of type-2 diabetes is a serious health issue worldwide. Its prevalence is associated with poor diet and unhealthy lifestyle choices, and it is characterised by high blood glucose levels that need to be controlled by medication. Metformin is the recommended and most effective first-line drug for type-2 diabetes but its use has also been linked to vitamin B12 deficiency, which increases the risk of peripheral nerve damage.

Aim and Objectives: The aim of the study is to estimate serum levels of Vitamin B12 levels in subjects with Type 2 Diabetes Mellitus. The objectives of the study include to estimate the prevalence of Vitamin B12 deficiency in Type 2 Diabetic subjects and to compare the serum levels Vitamin B12 in Type 2 Diabetic Subjects with or without metformin.

Materials and Methods: We included a total of 140 type 2 Diabetic subjects on metformin therapy for more than 3 years and 60 non-diabetics and non-metformin users. Serum levels of Vitamin B12 was estimated by Chemiluminescence Immuno Assay method. Serum Vitamin B12 levels of >300 pg/mL was defined as normal, 200-300 pg/mL insufficient and <200 pg/mL as deficient.

Results: We studied a total of 140 type 2 Diabetic metformin users and 60 non-metformin and non-diabetic subjects. We evaluated vitamin B12 levels in both. We found that 6.4% of metformin users had vitamin B12 deficiency, followed by 20.7% had insufficient Vitamin B12 levels and 72.8% had normal vitamin B12 status. Similarly, among non-metformin users 3.33% had deficient vitamin B12 levels, 16.3% had insufficient levels and 80% had normal vitamin B12 levels.

Discussion and Conclusion: Routine screening of Serum Vitamin B12 levels, complete hemogram, and neuropathy status should be done in all diabetic patients at the beginning of metformin therapy, and every 1-2 years thereafter. If any diabetic patient is found to be having low levels of Vitamin B12, they should be well supplemented with the Vitamin before start of metformin therapy.

Key-words: type 2 diabetes, metformin users, non-metformin users, vitamin B12 deficiency, vitamin B12 insufficiency.

INTRODUCTION:

Diabetes mellitus (DM) is a prevalent disease with multiple alarming complications that represent major threats to one's general health.[1] These complications are often associated with poor prognosis, increased morbidity, and impaired quality of life. Furthermore, the life span of patients with DM is dependent upon the presence/absence of these complications.[2]

The increasing incidence of type-2 diabetes is a serious health issue worldwide. Its prevalence is associated with poor diet and unhealthy lifestyle choices, and it is characterised by high blood glucose levels that need to be controlled by medication. Nerve damage in the periphery (e.g. face, limbs, organs) is a common complication of diabetes, with symptoms that range from numbness to pain, and can lead to debilitating loss of balance and co-ordination. Metformin is the recommended and most effective first-line drug for type-2 diabetes but its use has also been linked to vitamin B12 deficiency, which increases the risk of peripheral nerve damage. Despite the irreversibility of peripheral nerve damage, no official guidelines exist on screening vitamin B12 levels in patients treated with metformin.

Most patients tolerate metformin well, with most common side effects relating to GIT (nausea vomiting, diarrhea, lactic acidosis), which are usually mild and resolve with time. Vitamin b12 deficiency in DM induced by metformin therapy is least known side effect and is more likely with prolonged use of drug, higher doses taken and in elderly. Vitamin B12 is water soluble vitamin required for hematopoiesis, functioning of central and peripheral nervous system and in DNA synthesis of all cells. The reported Vitamin B12 deficiency in the general population of India varies from 12% to 67% as against that in western countries (5-7%).[3-6]

Hence we have taken up this study, to assess the prevalence of vitamin B12 deficiency in metformin users and to compare the vitamin B12 levels between metformin users and non-metformin users.

AIM AND OBJECTIVES:

Aim: The aim of the study is to estimate serum levels of Vitamin B12 levels in subjects with Type 2 Diabetes Mellitus.

Objectives: The objectives of the study include 1) to estimate the prevalence of Vitamin B12 deficiency in Type 2 Diabetic subjects and 2) to compare the serum levels Vitamin B12 in Type 2 Diabetic Subjects with or without metformin.

MATERIALS AND METHODS:

Place of Study: A prospective observational study on "Assessment of Vitamin B12 status in Type 2 Diabetic Subjects" was conducted at RIMS Raipur, in the department of Biochemistry from January 2021 to July 2021. **Study Population:**We included a total of 140 type 2 Diabetic subjectson metformin therapy for more than 3 years and 60 non-diabetics and non-metformin users.

Sample Size: 200

- 140 type 2 diabetic subjects on metformin therapy for more than 3 years.
- 60 non-metformin and non-diabetic subjects.

Inclusion Criteria: we included type 2 diabetic subjects on metformin therapy.

Exclusion Criteria:we excluded following subjects from our study

Subjects with history of pernicious anaemia,

Iron deficiency anaemia,

Inflammatory bowel disease,

Gastrointestinal surgeries,

Malnutrition,
h/o thyroid disorders,
Autoimmune disorders,
Peripheral arterial diseases,
Neuropathies due to other causes,
Patients on Vitamin B12, Vitamin D, Calcium supplementation, proton pump inhibitors and
Vitamin B complex for past 3 months,
Vegetarian,
Dementia and pregnancy
At baseline patients age, gender, duration of diabetes and dosage of metformin and usage of
other oral antidiabetic drugs were noted.

BloodSample Collection and Biochemical Investigations:5mL fasting venous blood was
drawn into plain red colored stoppered tube under aseptic precautions, blood allowed to clot
and centrifuged to obtain clear serum.Serum levels of Vitamin B12 was estimated by
Chemiluminescence Immuno Assay method. Serum Vitamin B12 levels of >300 pg/mL was
defined as normal, 200-300 pg/mL insufficient and <200 pg/mL as deficient.
Statistical Analysis: Microsoft Office Excel will be used for data storage, tabulation and
generation of descriptive statistics. Data were expressed in mean and SD. Students unpaired t
test will be used for the comparison. P value <0.05 will be considered statistically significant.

RESULTS: We included a total of 140 type 2 diabetic subjects and 60 non-diabetic and non-
metformin users after voluntary consent.

Table 1: Shows comparison of demographic factors and Vitamin B12 levels in Metformin and non-metformin users

	Metformin users	Non-metformin users	P value
N	140	60	
Age in years	49.8 ± 14.6	39.8 ± 11.6	HS
Gender Males Females	 80 60	 40 20	 S
BMI in kg/m^2	28.42 ± 5.96	25.41 ± 4.21	HS
Duration of DM (years)	8.42 ± 5.6	-	-
Systolic BP (mmHg)	134.42 ± 18.96	125.41 ± 17.21	HS
Diastolic BP (mmHg)	81.6 ± 13.62	78 ± 13.6	HS
Sulfonylureas	18	-	
Thiazide diuretics	09	-	
DPP4 Inhibitors	16	-	
Vitamin B12 Deficiency (<200) Insufficiency (200-300) Normal (>300)	 09 (6.4%) 29 (20.7%) 102 (72.8%)	 2 (3.33%) 10 (16.3%) 48 (80%)	 S S -

It is quite evident that the metformin users had significantly decreased vitamin B12
levels as compared to non-metformin users.

DISCUSSION: We studied a total of 140 type 2 Diabetic metformin users and 60 non-
metformin and non-diabetic subjects. We evaluated vitamin B12 levels in both. We found
that 6.4% of metformin users had vitamin B12 deficiency, followed by 20.7% had

insufficient Vitamin B12 levels and 72.8% had normal vitamin B12 status. Similarly, among non-metformin users 3.33% had deficient vitamin B12 levels, 16.3% had insufficient levels and 80% had normal vitamin B12 levels. The overall prevalence of vitamin B12 deficiency was significantly more in metformin users as compared to non-metformin users. Metformin treatment in diabetic women is associated with higher prevalence of Vitamin B12 deficiency. Deficiency of Vitamin B12 increases with duration of DM and dose of metformin. Vitamin B12 deficiency did have an impact on development of macrocytic anemia and worsening of neuropathy.Some symptoms of vitamin B12 deficiency are difficult to diagnose and can be irreversible if left untreated (like neuropathy). On the other hand, treatment of vitamin B12 deficiency is relatively easy, cheap, safe, and effective.

The findings our study were similar to that of other studies i.e one early randomized control trial by DeFronzo et al, metformin decreased the serum vitamin B12 levels by 22% and 29% compared to placebo and glyburide respectively. Metformin use has been unequivocally demonstrated as the prime factor associated with vitamin B12 deficiency among patients with T2DM.[7-11]

Vitamin B_{12} deficiency is a multifactorial condition caused by insufficient intake (nutritional deficiency) as well as acquired or inherited defects that disrupt B_{12} absorption and processing pathways. Similarly, metformin-induced B_{12} deficiency is also thought to occur due to vitamin B_{12} malabsorption such as alteration of bile acid metabolism, small intestinal bacterial overgrowth, or effects on intrinsic factor secretion, but a more currently accepted explanation is the interference by metformin on calcium-dependent membrane action responsible for vitamin B_{12} intrinsic factor absorption in the terminal ileum. The use of PPIs is also thought to contribute to B_{12} deficiency, although this does not appear to be a factor in our study. Both observational and interventional studies have shown that the duration and dose of metformin are also associated with B_{12} deficiency and neuropathy.A recent study from Qatar, however, showed no association between metformin use and B_{12} deficiency or diabetic neuropathy.

Groot-Kamphuis *et al* have shown a lower prevalence of DPN in people with T2DM on metformin compared with those not on metformin.[31] Our study confirms a weak but significant correlation between B_{12} levels and duration and dose of metformin. A significant association has also been found with age, gender, married individuals, BMI and blood pressure with B_{12} levels in metformin users.[11-14]

CONCLUSION:

Routine screening of Serum Vitamin B12 levels, complete hemogram, and neuropathy status should be done in all diabetic patients at the beginning of metformin therapy, and every 1-2 years thereafter. If any diabetic patient is found to be having low levels of Vitamin B12, they should be well supplemented with the Vitamin before start of metformin therapy. Another approach is to compulsorily administer 1000μg of Vitamin B12 injection once a year in all diabetic patients on metformin therapy.

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