Vitamin B_{12} Deficiency in Type 2 Diabetes Patients on Metformin

A Cross-sectional Survey from South India

ABSTRACT

Type 2 Diabetes mellitus is a chronic metabolic disorder with complications of microangiopathies, macroangiopathies, neuropathy and nephropathy. Metformin is often the first line treatment in type 2 diabetes. There is evidence of a higher prevalence of vitamin B_{12} deficiency observed among patients on metformin. It is believed that Metformin may cause vitamin B_{12} malabsorption which aggravates neuropathy and anaemia.

Methods

A cross-sectional study was designed with randomly selected type 2 diabetes patients attending a tertiary diabetes clinic in Southern India, recruited over a period of 3 months, divided in 2 groups (control and study groups) based on their use of metformin. They were assessed for serum B_{12}, Folic acid levels and mean corpuscular volume. Clinical manifestations of neuropathy, anaemia and vitamin B_{12} deficiency were documented on clinical examination.

Results

There were 100 patients recruited, showing those on metformin had a higher prevalence of vitamin B_{12} deficiency (58.3% vs 33.3%, $p<0.05$), however, folic acid deficiency was lower (16.7% vs 37.5%, $p<0.05$).

Conclusion

Patients with type 2 diabetes on metformin required protocols for monitoring and replacement of vitamin B12 as necessary, to prevent onset of neuropathy.

KEYWORDS:

Metformin, vitamin B_{12} deficiency, type 2 diabetes mellitus

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INTRODUCTION

Type 2 Diabetes mellitus[1] is the leading metabolic disorder in the world and is a major cause of blindness, lower limb amputations, stroke, heart and kidney failure in developing countries. Metformin is the first line drug prescribed for the disease and is shown to reduce development of diabetes over 15 years, as does lifestyle intervention.[2] However, there is evidence suggesting that metformin may cause vitamin B_{12} and homocysteine deficiency.[3]

Vitamin B_{12} has an important role in cellular metabolism, especially in DNA synthesis, methylation and mitochondrial metabolism. Clinical vitamin B_{12} deficiency with classic haematological and neurological manifestations is relatively uncommon. However, subclinical deficiency affects between 2.5% and 26% of the general population depending on the definition used, although the clinical relevance is unclear. B_{12} deficiency can affect individuals at all ages, but most particularly elderly individuals. Infants, children, adolescents and women of reproductive age are also at high risk of deficiency in populations where dietary intake of vitamin B_{12}-containing animal-derived foods is restricted. Deficiency is caused by either inadequate intake, inadequate bioavailability or malabsorption. Disruption of vitamin B_{12} transport in the blood, or impaired cellular uptake or metabolism causes an intracellular deficiency.

High prevalence of low B12 levels has been shown in European (27%) and South Indian (32%) patients with type 2 diabetes.[4] Risk factors that predispose Indians to vitamin B_{12} deficiency include vegetarianism,[5] reduced intake of animal liver among non-vegetarians, usage of proton-pump inhibitors (PPI's)[6], mal-absorptive diseases and restricted availability of food due to socio-economic deprivation particularly among pregnant women[7], [8] and the older populations.[9]

Interaction with Metformin

Diagnostic biomarkers for vitamin B_{12} status include decreased levels of circulating total vitamin B_{12} and transcobalamin-bound vitamin B_{12}, and abnormally increased levels of homocysteine and methylmalonic acid. Several hypotheses have been proposed to explain the causative mechanism of metformin-induced vitamin B12 deficiency. The most accepted mechanism proposes calcium interference by metformin. Calcium aids the Intrinsic Factor-B_{12} complex bind to cubulin receptors present in ileal cells.[10] It is proposed that protonated metformin reaches out to the hydrocarbon core of ileal cell membrane, thereby positively charging the surface and displacing calcium ions. Thus absence of calcium at ileocytes may account for B_{12} malabsorption. [11]

Vitamin B12 deficiency leads to peripheral neuropathy (PN) and damage to spinal cord and demyelination of the dorsal column. Neuropsychiatric manifestations include cognitive Impairment [12] and clinical depression.[13] On the other hand, symptoms such as delirium, altered sensorium and disorientation can be a result of hypoglycaemic state or diabetic ketoacidosis. Haematological manifestations include macrocytic anaemia. A study from Northern India, reported a symptomatic vitamin B_{12} deficiency prevalence of 27-59%, particularly in strict vegetarians (86%), those with diabetes[14] and malabsorption. [15] Dietary supplementation of vitamin B12 in urban older adults and pregnant women may have a positive impact on prevalence of deficiency. [8],[16]

We were keen to explore the prevalence of

vitamin B12 deficiency in our cohort of type 2 diabetes patients in South India, considering the lifestyle factors and understand whether our local protocols should include testing, monitoring and replacement.

METHODS

We designed a cross-sectional study to assess prevalence of Vitamin B_{12} deficiency and its clinical manifestations in patients with type 2 diabetes on metformin. This study was conducted in the outpatient clinic of the Department of Endocrinology at a tertiary care hospital in South India. Ethical permission was granted by the Institutional research and ethics committee. We invited a randomised cohort of subjects with type 2 diabetes mellitus between 45 and 75 years to participate in the study. Written consent was obtained.

Both test and control groups contained 48 subjects based on sample size calculations.[17] The cases group consisted of patients with type 2 diabetes on metformin for >18 months. While control consisted of patients with type 2 diabetes not on metformin and creatinine levels <2mg/dl. Patients with small bowel disorders, atrophic gastritis, pregnancy, and those on vitamin B_{12} or Folic acid supplements were excluded.

We assessed history of manifestations of vitamin B_{12} deficiency using questionnaires. Manifestations such as peripheral neuropathy, cognitive impairment and glossitis seen in diabetics and anaemia was recorded by performing clinical examinations on both groups and later compared based on the severity. Serum vitamin B_{12} and folic acid status was assessed using chemiluminescent immunoassay (CLIA).

RESULTS

This study included 96 participants out of which, 56% (54) were females and 43.8% (42) were males. Among both groups, ages ranged between 45-75 and the mean age was found to be 52 years. Vitamin B12 levels were found to be deficient among 47.6% of males and 47.1% of females. While folic acid deficiency was detected in 33.3% males and 22.2% females.

Dietary Influence

Majority of the study population were non-vegetarians (86.5%) while only 13.5% were vegetarians. Vitamin B12 deficiency was found among 66.7% vegetarians and 44% non-vegetarians and was statistically not significant. 15.4% vegetarians and 28.9% non-vegetarians had folic acid deficiency.

The population characteristics are presented in table 1 and the mean/median of serum vitamin B12, folic acid levels and mean corpuscular volume is presented in table 2.

Table 1: Comparison of baseline characteristics between the two groups.

PARAMETER	Cases	Controls	**P value**
SEX A. Male B. Female	 33.3% 63%	 66.7% 37%	0.004
DIET A. Vegetarian B. Non-Vegetarian	 53.8% 49.4%	 46.2% 50.6%	0.765
NEUROPATHY	50.8%	48.6%	0.834
FOOT ULCERS	75%	25%	0.307
GLOSSTIS	33.3%	66.7%	0.557
BMI A. NORMAL (18.5-24.9) B. OVERWEIGHT (25-30.9) A. OBESE(>31)	 5.4% 52.9% 41.7%	 5.8% 45.1% 49.1%	0.072
VITAMIN B12 A. NORMAL B. BORDERLINE A. DEFICIENT	 33.3% 16.2% 50.5%	 60.4% 13.2% 26.4%	0.025
FOLIC ACID A. Normal B. Deficient (<10ng/ml)	 67.1% 32.9%	 42% 58%	0.022
MCV A. Normal (79.9-100fl) B. Deficient (<79.9fl)	 39.5% 60.5%	 54.9% 45.1%	0.203
ANAEMIA Male (<13gm/dl) Female (<12gm/dl)	43.8%	56.2%	0.036
T2DM DURATION A. >=10 years B. <10 years	 55% 45%	 40% 60%	0.000
HB_{A1C} (<7)	41.0%	34.3%	0.551
SERUM CREATININE A. >=2mg/dl B. <2mg/dl	 51.4%	 48.6%	

Table 2: Mean and median of serum vitamin B_{12}, serum folic acid and MCV in users and non-user.

Biochemical parameters	**Cases**	**Controls**	**P value**
Vitamin B_{12} **1.** Median **2.** Mean (SD)	 168 356.40 (1062.76)	 263.5 302.29 (177.82)	0.025 **(S)**
Folic Acid **1.** Median **2.** Mean (SD)	 28.45 34.74 (31.873)	 11.00 14.05(15.182)	0.022**(S)**
MCV **1.** Median **2.** Mean (SD)	 82.85 97.99 (118.81)	 83.72 84.75(4.89)	0.203**(NS)**

We found a significantly higher prevalence of vitamin B_{12} deficiency in patients on metformin, (60.9% vs 36.4%, p=0.002) while folate deficiency was more prevalent in the control group (69.2% vs 30.8%, p=0.022), figure 1.

Figure 1: Serum Vitamin B_{12} and serum Folic acid – Proportions

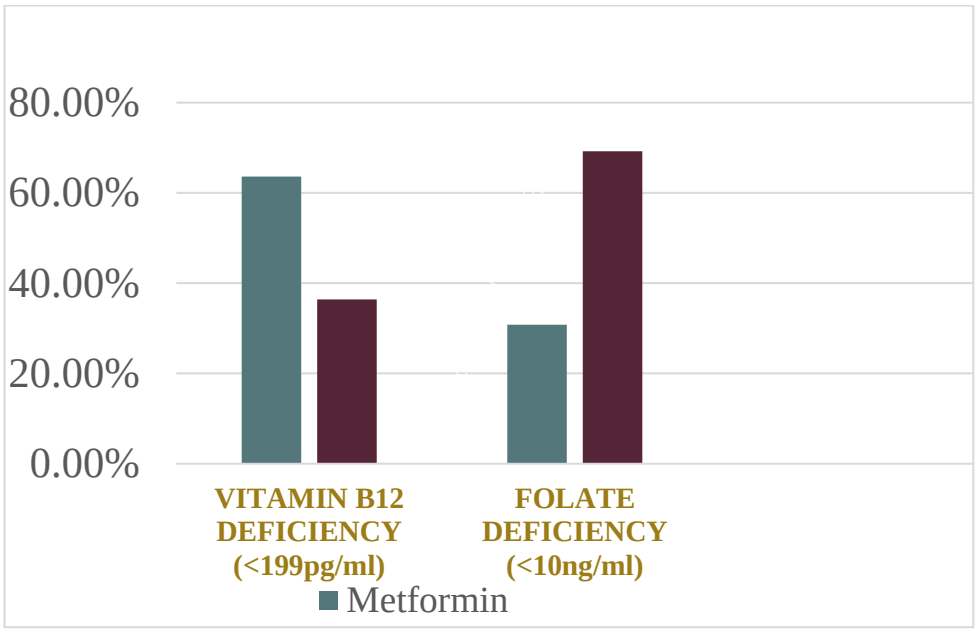

Table 3: Comparison of Folic Acid levels among background parameters

PARAMETER	NORMAL	DEFICIENT	P VALUE
SEX			
A. male	66.7%	33.3%	0.224
B. female	77.8%	22.2%	
DIET			
A. vegetarian	84.6%	15.4%	0.307
B. non-vegetarian	71,1%	28.9%	
SUBSTANCE ABUSE	56.5%	43.5%	0.042
INSULIN PEN USAGE	70.6%	29.4%	0.812
NEUROPATHY	67.6%	32.4%	0.350
PALLOR	86.7%	13.3%	0.041

DISCUSSION

Among metformin users, there is a negative correlation between duration of metformin usage and Vitamin B_{12} levels. This finding is concurrent with that of Kumar et al [(1)] and that of Agarwal et al which found significant reduction of B12 with increased number of years on Metformin. On the other hand, normal folic acid levels were seen in majority of patients who were on the drug. The high incidence of users with normal folic acid levels maybe explainable by the folate trap cycle. The study included patients who were uniformly prescribed 1000 mg Metformin and hence was incapable of assessing the impact of metformin dose on B12 and Folic acid levels.

In this study, it was found that a higher proportion of patients on Metformin had serum Vitamin B_{12} deficiency, compared to controls. It should be noted that Vitamin B_{12} has a significantly higher median value among controls due to a skewed value in the group.

On the contrary, folic acid deficiency was more pronounced among controls than cases. The possible explanation for higher folic acid levels and deficient B12 levels among metformin users may be the folate trap cycle. Due to lack of B_{12}, 5-methyl-Tetrahydrofolate is not converted to Tetrahydrofolate (THF) which leads to its increased serum levels. At the same time, B_{12} deficiency also leads to hyperhomocystinemia[11] that can lead to serious cardiovascular and neurological sequelae [13]. Hence, one might consider using MMA and Homocysteine levels as deficiency markers, however they have proved to be expensive and unreliable in some experiments[14]. The study also used MCV as a possible marker for B_{12} deficiency but did not find significant differences between both groups. Paradoxically, haemoglobin was found to be higher among metformin users than non-users.

Clinically, this study attempted to assess vitamin B_{12} deficiency complications and found an increased incidence of neuropathy (tingling and burning sensation of extremities) and foot ulcers among cases. Another study showed strong correlation between worsening neuropathy and cumulative Metformin dose, stating that the drug in fact aggravates the already existing diabetic neuropathy[15]. Hence, one must periodically check for exacerbated

signs and symptoms. On the contrast, Kumar *et al*[1] and Carmel R *et al*[4] reported that neuropsychiatric symptoms are not well pronounced among those with biochemical B_{12} deficiency. In this study, anaemic glossitis was additionally studied and was found to be more prevalent among controls than cases. Therefore, clinical manifestations may not be reliable in all cases of B_{12} deficiencies and must be correlated with their biochemical values.

An audit on vitamin B_{12} deficiency in Indian patients taking metformin is scarce. This specific population majorly suffers from the deficiency due to vegetarianism, common use of proton pump inhibitors, heavy intake of antibiotics, low calcium/vitamin D levels due to sedentary lifestyle. It should be noted that, this study did not find significant difference of B_{12} levels among vegetarians and non-vegetarians. This may be due factors like non-consumption of B_{12} rich animal liver or variability in the amount of meat consumption. Therefore, irrespective of dietary contribution, Indian diabetics on Metformin should be timely screened for B_{12} biochemical deficiencies along with signs of aggravated neuropsychiatric manifestations and ischemic cardiac changes. Studies on serum Folic acid levels in Metformin prescribed diabetics are less and almost nil in India. Apart from routine Vitamin B_{12} supplementation, this study has found no need of folic acid supplementation as its levels are optimum.

CONCLUSION

Among type 2 diabetic patients, vitamin B_{12} deficiency is more common among Metformin users and the number of years of use is directly proportional to it is level of deficiency. For the same reason, B12 and calcium supplementation is recommended when prescribing Metformin. Unlike the previous studies, this study revealed that folate is higher among the metformin users explainable by the folate trap theory. Hence, folic acid supplements are not necessary. Contrary to the common belief, non-vegetarianism has little effect on serum vitamin B_{12} levels. This study also demonstrated that clinical signs of anaemia and neuropathy because of vitamin B_{12} deficiency are non-reliable, thus serum vitamin B_{12} assay is necessary to establish its deficiency.

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