Review Article

Metformin Induced Vitamin B_{12} Deficiency among Type 2 Diabetes Mellitus Patients

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ABSTRACT

Metformin is the most frequently prescribed medication in the management of Type 2 Diabetes Mellitus. It is widely approved that it suppresses hepatic glucose production and improves insulin signalling mainly in muscle, hepatic and adipose tissue. On long term use, metformin therapy leads to Vitamin B_{12} deficiency and anemia. Several studies shows that long term metformin use reduce the Vitamin B_{12} levels and particularly taken in a dose greater than 2000 mg/day and for a period exceeding 4 years. The prevalence is increased with increase in dose and duration of metformin use. Peripheral neuropathy may be the only clinical presentation of Vitamin B_{12} deficiency, without haematological signs and symptoms. The diagnostic tests like serum Vitamin B_{12} and holo -TC-11 test measure the circulating part of Vitamin while homocysteine and MMA are the biomarkers of metabolic Vitamin B_{12} deficiency that show elevated levels when the Vitamin is deficient at the cellular level. Currently there are no guidelines for the supplementation and appropriate dose of Vitamin B_{12} for diabetic patients on metformin but the treatment of Vitamin B_{12} deficiency includes monthly injections of Vitamin B_{12} or large daily therapeutic doses (1000mcg) of Vitamin B_{12}, prophylactically administered calcium carbonate (1.2gms daily). This article demonstrates that regular monitoring of Vitamin B_{12} should be done especially in patients receiving metformin therapy for longer duration at high dosage and Vitamin B_{12} supplementation prophylactically or at least annually to prevent the complications of Vitamin B_{12} deficiency.

Key words: Type 2 Diabetes Mellitus, Metformin, Homocysteine, Vitamin B_{12} deficiency, Peripheral neuropathy.

INTRODUCTION

Metformin, an oral hypoglycaemic agent is the most frequently prescribed medication in the management of Type 2 Diabetes Mellitus (T2DM). All guidelines, including the European Association for the Study of Diabetes (EASD) and American Diabetes Association (ADA) focus on metformin as the first line treatment option along with lifestyle intervention for hyperglycaemic management in T2DM.[1] Metformin may also be used to treat other conditions involving insulin resistance and polycystic ovary syndrome (PCOS).[2]

Metformin is widely approved that it suppress hepatic glucose production and improves insulin signalling mainly in muscle, hepatic and adipose tissue.[3,4] The main side effects of metformin include GI disturbance such as diarrhoea and vomiting, hypoglycaemia and lactic acidosis.[5,6] On long term use, metformin therapy leads to Vitamin B_{12} deficiency and anemia.[7]

Vitamin B_{12} is a vital nutrient for health. It plays an important role in the functioning of the brain and nervous system and in the formation of red blood cells. In addition to anemia, Vitamin B_{12} deficiency may increase the severity of peripheral neuropathy in patients with T2DM.[8] Furthermore, because Vitamin B_{12} participates in most important pathway of homocysteine (Hcy) metabolism, a reduction in Vitamin B_{12} would increase plasma concentrations of Hcy, which is strongly linked to cardiovascular disease in patients with T2DM[9] and PCOS.[10] This review aims to describe the Vitamin B_{12} deficiency induced by metformin among T2DM patients and it importance to prevent further complications by Vitamin B_{12} supplementation.

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History and Background

Metformin, a cornerstone medication, used to manage T2DM with estimates which is routinely prescribed to 120 million diabetic patients around the world.[11] In 1971, it was surprising knowing that the first article (Tomkin *et al*.) describes metformin associated with Vitamin B_{12} malabsorption.[12]

Despite the confirmed association between metformin and Vitamin B_{12} deficiency, the real size of the problem is not yet properly quantified. Previous studies have shown that the prevalence of metformin induced Vitamin B_{12} deficiency varied greatly and ranged between 5.8% and 52%. Such a wide range may be attributed to difference in cut points chosen to define the deficiency, participants mean age, study setting, metformin dose and duration of use.[13]

Peripheral neuropathy may be the only clinical presentation of Vitamin B_{12} deficiency, without haematological signs and symptoms. The long term use of metformin, mediated by Vitamin B_{12} deficiency, may contribute to increasing the substantial burden of peripheral neuropathy in T2DM patients.[14]

Prevalence of Vitamin B_{12} deficiency among T2DM patients

Several studies shows that long term metformin use reduce the Vitamin B_{12} levels and particularly taken in a dose greater than 2000 mg/day and for a period exceeding 4 years. Comparing the obtained prevalence of metformin associated Vitamin B_{12} deficiency from earlier epidemiological studies is not straightforward and should consider several factors. Moreover, the biomarkers used to define the deficiency, together with their cut-offs, can greatly affect the value of prevalence estimate.

Table 1 shows the prevalence estimates and certain characteristics of the studies that used Vitamin B_{12} deficiency cutoff points of 148 or 150 pmol/L. The table reveals study-related factors with potential to affect the obtained prevalence, including mean participants age, mean metformin daily dose, study settings, mean metformin duration of use and whether participants with renal impairment were excluded. Special attention should also be paid to the mean ages in different studies as Vitamin B_{12} levels decreases with age. Variations in doses and durations of metformin use can also impact the final prevalence values.

Effect of metformin on Vitamin B_{12} level

Metformin prevents the absorption of Vitamin B_{12} in the ileum and this is caused by inhibition of calcium dependent channels in the ileum. It is known that prolonged use of metformin cause Vitamin B_{12} deficiency by this mechanism.[15]

Ko S-H *et al*. shows patients with Vitamin B_{12} deficiency had a longer duration of metformin use ($p<0.001$), a larger daily dose of metformin ($p<0.001$) than the patients without Vitamin B_{12} deficiency.[16] There was a significant lower Vitamin B_{12} concentrations among those patients receiving 1000mg/day to 2000mg/day than those receiving 1000mg.

DeJager *et al*. provided the strongest evidence of metformin associated low Vitamin B_{12} levels by conducting 4.3 years duration randomized controlled trial. The trial reported a 19% metformin associated reduction in Vitamin B_{12} levels.[17]

Liu Q *et al*. a meta-analysis also confirmed that metformin induces a reduction inVitamin B_{12} levels. This study reported the positive association between the metformin dose and the lowering of the Vitamin concentrations.[18]

Diagnosis of Vitamin B_{12} deficiency

The diagnostic tests like serum Vitamin B_{12} and holo -TC- 11 test measure the circulating part of Vitamin while homocysteine and MMA are the biomarkers of metabolic Vitamin B_{12} deficiency that show elevated levels when the Vitamin is deficient at the cellular level. The more accurate biomarkers have their own sensitivity and specificity limitations.[19]

Serum Vitamin B_{12} test

The sensitivity of the serum Vitamin B_{12} test in assessing the Vitamin status is generally high. Several studies shows that Vitamin B_{12} levels <148 pmol/L have a sensitivity that exceeds 95% in patients with megaloblastic anemia.[20] Bolann *et al*. used >50% post-therapy decline in MMA as a gold standard to define Vitamin B_{12} but the specificity of serum Vitamin B_{12} test is low.[21] Clarke *et al*. applied strict MMA criteria of >450 and >750 nmol/L as reference tests and found that serum Vitamin B_{12} <200 pmol/L had specificities of 72% and 75% respectively. The low serum Vitamin B_{12} levels were falsely reported in pregnancy and folate deficiency.[22]

Jeffery *et al*. reported that high TC-I levels account for 8% of cases with elevated serum Vitamin B_{12} levels. People of black ethnicity tend to show higher circulatory levels of TC-I and Vitamin B_{12}. The Vitamin B_{12} concentrations are elevated also in renal disease patients.[23]

Table 1: Clinical studies measured the prevalence of metformin – Vitamin B_{12} deficiency with their diagnostic cut-off points of 148 or 150 pmol/L and other sample and study characteristics.

Study	Obtained prevalence	Mean age (years)	Mean metformin dose (mg)	Mean metformin duration (years)	Study settings	Exclusion of renally-impaired patients
DeJager *et al.*	9.9%	64	2050	4.3	Outpatient clinics, the Netherlands	Yes
Reinstatler *et al.*	5.8%	63.4	NA	5*	NHANES, United States	Yes
Hermann *et al.*	8%	58.2	2200	5.2	Outpatient clinic, Sweden	Yes
Liu *et al.*	29%	79.7	NA	NA	Geriatric outpatient clinic, Hong Kong	No
Beulens *et al.*	28.1%	61.6	1306	5.3	Primary care centre, the Netherlands	No
DeGroot-kamphuis *et al.*	14.1%	62.6	NA	4.9*	Outpatient clinic, the Netherlands	No
Ahmed *et al.*	28.1%	58.5	2400	9.6	Outpatient diabetes clinics of 2 tertiary hospitals, South Africa	Yes

*median value; NHANES: National Health and Nutrition Examination Survey

MMA Test

Vitamin B_{12}, under the catalysis of the enzyme methylmalonyl-CoA mutase, synthesizes succinyl-CoA from methylmalonyl-CoA in the mitochondria. Deficiency of Vitamin B_{12} thus results in elevated MMA levels. Thus measuring MMA levels provides a more accurate estimation of the cellular status of Vitamin B_{12} compared with the Vitamin's serum levels. Elevated MMA test has >95% sensitivity to Vitamin B_{12} deficiency in patients with pernicious anemia.[24] In such overt deficiencies, sensitivity of MMA elevation is slightly better than that of low Vitamin B_{12} levels.[25]

Wile DJ *et al.* a case control study reported higher MMA levels in T2DM patient who were taking metformin compared to the group not taking metformin and also reported a correlation between cumulative dose of the medication and MMA levels for the first time.[8]

Pfeiffer *et al.* used the low cut off point of 210 nmol/L as a physiologic choice based on MMA levels in Vitamin B_{12} depleted individuals. Usually MMA test cut offs ranging between 210 and 480 nmol/L are used to define Vitamin B_{12} deficiency. This represents there is a maximal inhibition of MMA levels by administering Vitamin B_{12}.[26]

The antibiotics have the ability to reduce MMA levels suggests a role for the gut bacteria that produce propionic acid, the precursor of MMA.[27] Therefore, the specificity of the MMA test is uncertain and the test is not qualified for use as a gold standard for defining Vitamin B_{12} deficiency.

HOLO TC II Test

Vitamin B_{12} circulates in plasma bound to TC I (70-80%) and TC II carrier proteins (20-30%) to form a metabolically inert complex. The portion attached to TC II protein is known as holo-TC II. Chen *et al.* found that the metabolic status of Vitamin B_{12} was a major determinant of holo-TC II serum levels and also concluded that the absorption status of Vitamin B_{12} are influenced by serum holo-TC II levels.[28]

Several studies suggested that the levels of holo TC II are affected by folate disorders, use of oral contraceptives, myelodysplasia, certain haematological disorders and alcoholism.[29-31] Mild renal insufficiency has a modest impact on serum Vitamin B_{12} and holo-TC II levels unlike its effect on MMA and homocysteine concentrations.[32]

Homocysteine test

The MS enzyme catalyses the transfer of a methyl group from methyl-tetrahydrofolate to homocysteine to result in the formation of tetrahydrofolate and methionine, utilizing Vitamin B_{12} as a cofactor. Thus, elevated homocysteine concentrations are associated with Vitamin B_{12} deficiency and homocysteine may be used as a test to assess Vitamin B_{12} metabolic status.

McPartlin J *et al.* recommends setting cut offs for homocysteine levels by considering age and folate fortification status. For folate fortified communities, it recommends 12 micromol/L and 16 micromol/L for those aged 15-20 micromol/L for those aged 15-65 years and >65 years, respectively if not folate fortification implemented, the cut-offs of 15 and 20 micromol/L was recommended.[33]

Metformin users where found to have slightly higher homocysteine levels than non-users.[34] De Jager *et al.* a randomized controlled trial of 4.3 years treatment with metformin resulted in a minor statistically significant increase in homocysteine concentrations.[17]

Falsely positive renal failure, old age, Vitamin B_6 and Vitamin B_2 deficiencies can also cause increased homocysteine concentrations.

Clinical manifestations of Vitamin B_{12} deficiency

Vitamin B_{12} deficiency is clinically important because it is a reversible cause of bone marrow failure and demyelinating nerve disease. Thus haematological manifestations include macrocytosis and megaloblastic anemia which may be associated with other signs and symptoms of deficiency such as pancytopenia, glossitis, gastrointestinal dysfunction, psychosis or neurological disorders.[35] Neurological signs and symptoms may take many forms, including peripheral neuropathy which generally manifests as numbness and paresthesia,[36] optic neuropathy[37] and neuropsychiatric disorders such as chronic fatigue syndrome, mood disorders or depressive symptoms.[38]

Vitamin B_{12} deficiency may also result in improper bowel motility, which manifests as mild constipation or diarrhoea and loss of bowel or bladder control may develop.[39] The deficiency may impair immune response and low bone mineral density.[40]

Neuropathic pain from Vitamin B_{12} deficiency should be differentiated from that of diabetic neuropathy. So diabetic neuropathy can be confirmed by electromyography or nerve conduction tests.

Clinical consequence of metformin induced Vitamin B_{12} deficiency

Peripheral neuropathy is a primary complication of T2DM and a direct manifestation of Vitamin B_{12} deficiency. It was recently investigated by five observational studies with conflicting results. Three studies reported no association; two reported increased neuropathy among metformin-exposed patients.[8,13,39-42.]

Neuropsychiatric manifestations such as depression and cognitive impairment were linked with low Vitamin B_{12} levels. Two recent studies reported that the Vitamin B_{12} deficiency among metformin treated patients was associated with worsened cognitive performance and increased risk of depression.[43,44]

But none of the studies on metformin associated low Vitamin B_{12} have not reported any significant impact on haematological findings like Haemoglobin concentrations, prevalence of anaemia, mean corpuscular volume or macrocytosis as a primary objective.[45,46]

Management of Vitamin B_{12} deficiency

Currently there are no guidelines for the supplementation and appropriate dose of Vitamin B_{12} for diabetic patients on metformin. Treatment of Vitamin B_{12} deficiency includes monthly injections of Vitamin B_{12} or large daily therapeutic doses (1000 mcg) of Vitamin B_{12}, prophylactically administered calcium carbonate (1.2 gms daily).[47] In severe cases, discontinuation of metformin therapy is recommended.[48] The dosing pattern of Vitamin B_{12} depends on cause of the deficiency and the severity of the disease.[46] Vitamin B_{12} can be supplemented in various forms like hydroxocobalamin, methylcobalamin and cyanocobalamin. However studies have shown that methylcobalamin is better retained in the body than its cyanide containing sibling, cyanocobalamin. Multivitamin use is convenient, non-invasive, inexpensive and generally effective in increasing serumVitamin B_{12} concentrations but it is insufficient for diabetic patients taking metformin. Kancherla *et al.* found that patients receiving metformin therapy who also used oral multivitamin supplements had a 50% higher serum Vitamin B_{12} or about 161 pmol/L higher serum concentrations, compared to those patients who did not use multivitamin supplements.[49] Only 4% of those taking multivitamin supplements had sub normal Vitamin B_{12} concentration compared with 15% among non-multivitamin supplement users. On the other hand, Reinstatler *et al.* concluded that 6 mcg per day of Vitamin B_{12} found in most multivitamin supplements is insufficient.[50] Diabetic patients who ingested less than 6 mcg per day of Vitamin B_{12} from supplements had nearly 8 times higher risk of deficiency of this Vitamin compared to those who ingested a dose greater than 25 mcg per day or higher. Thus, a long term use of oral Vitamin B_{12} supplementation a dose of 25 mcg per day might be needed to maintain adequate Vitamin B_{12} status among these individuals, patients who use other medications such as aspirin or those that affect gastric acidity may needed to utilize supplements with higher doses (E.g. 100 mcg or 250 mcg).[51] The same may be true of elderly patients with diabetes.

CONCLUSION

Vitamin B_{12} deficiency occurs more frequently in patients with type 2 diabetes with longer duration of metformin use and in those taking larger amounts of metformin. Several studies have recently investigated that metformin

induced Vitamin B_{12} deficiency ability to cause or worsen peripheral neuropathy in T2DM patients and the high prevalence obtained with increase in dose and duration. This article demonstrates that regular monitoring of Vitamin B_{12} should be done especially in patients receiving metformin therapy for longer duration at high dosage and Vitamin B_{12} supplementation prophylactically or at least annually to prevent the complications of Vitamin B_{12} deficiency.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS

T2DM: Type 2 Diabetes Mellitus; **PCOS:** Polycystic ovary syndrome; **Hcy:** Homocysteine; **MMA:** Methylmalonic acid; **HOLO TC II:** Vitamin B_{12} bound transcobalamin-II.

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