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A Brief Review on the Association of Prolonged Metformin Therapy and Vitamin B12 Deficiency

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ABSTRACT

Type 2 Diabetes Mellitus is a fast-growing health challenge, signaled by hyperglycemia. There are two types of diabetes mellitus, Type 1 and Type 2; both are equally life threatening condition. Type 1 diabetes is a persistent autoimmune disease as a consequence of insulin deficiency, whereas in Type 2 diabetes the body either does not produce enough insulin or develops resistance to insulin. Metformin is the exclusive pharmacologic management for Type 2 diabetes mellitus and frequently prescribed drug either single or in combination with insulin or other hypoglycemic agents. Metformin has immensely improved insulin sensitivity and defend against vascular complications. Vitamin B 12 (cobalamin) has a vital role in DNA synthesis, cellular metabolism and the deficiency will lead to megaloblastic anemia. Several studies state that metformin can cause cobalamin deficiency in doses greater than 2000 mg/ day and for a time period of more than 4 years. Hence, cobalamin and calcium supplementation will reduce the occurrence of cobalamin deficiency in patients with metformin therapy.

KEY WORDS: Type 2 diabetes mellitus, Metformin, Vitamin B 12, Vitamin B 12 deficiency, Cobalamin.

INTRODUCTION

Type 2 Diabetes Mellitus is a serious metabolic disease; there are two types of diabetes, Type 1 and Type 2. Type 1 diabetes is a persistent autoimmune disease marked by insulin deficiency. T cell mediated destruction of beta cells in the pancreas is the major cause of insulin deficiency. The pathogenesis of Type 1 Diabetes Mellitus also includes the presence of HLA-DR/DQ alleles, environmental triggers and food antigens, which lead to an autoimmune process. Type1 Diabetes Mellitus is more found in children and adolescents. But in its present condition, it can develop at any age and may experience notable weight loss, Polydipsia, Polyuria and Polyphagia. In Type 1 DM, people generally present with extreme hypoglycemia. The treatment pattern of Type 1 DM is the use of Insulin. Target model therapy and transplantation are also used to manage Type 1 DM; they embrace Insulin gene therapy, Whole pancreas transplant, Islet cell transplant and Stem cell therapy [1].

Type 2 diabetes mellitus is signalized by hyperglycemia, which occurs as a result of insulin deficiency. People with Type 2 diabetes mellitus are more exposed to different forms of complex conditions. The

origin or cause of Type 2 Diabetes Mellitus related to genetic factors like insulin secretion, insulin resistance and environmental factors. The pathophysiology behind the development of Type 2 diabetes mellitus is marked by these causes. The role of environmental factors has an excessive role in the development of diabetes mellitus. These include diet, age, obesity, alcohol consumption, smoking, etc. [2]

The pharmacological management of Type 2 DM is complex and needs agents have different action because of the uneven pathophysiological changes behind the development of Type 2 DM. The management of Type 2 DM is targeted to prevent the micro and macro vascular complications. The treatment should achieve Hemoglobin A1C <7%, blood pressure <130/80 mm Hg and cholesterol should be < 2.6 mmol/L. A wide range of oral hypoglycemic drugs and injectable insulins are available for the treatment. Of these drugs, Metformin is most commonly prescribed. The mechanism of Metformin is inhibition of hepatic gluconeogenesis and promotion of insulin signaling in adipose tissues and muscles. The major side effects of metformin are diarrhea, vomiting and lactic acidosis. Furthermore, long term use of metformin can cause Vitamin B 12 deficiency. Vitamin B 12, also known as Cobalamin, which have crucial role in DNA synthesis, formation of blood cells and functioning of the brain. Low cobalamin will give rise to peripheral neuropathy. Furthermore, it influences homocysteine pathway; low cobalamin would shoot up plasma homocysteine levels, which are connected with cardiovascular diseases [3]. This review is intended to sketch the Metformin induced Vitamin B 12 deficiency and the precautions to prevent further complications.

METFORMIN

Currently, Metformin is the first line and widely prescribed oral hypoglycemic drug. Guidelines, including European Association for the Study of Diabetes (EASD) and American Diabetes association, pointed out Metformin as the first choice therapy together with dietary and lifestyle changes [4]. Metformin is a dimethylbiguanide class of anti-diabetic drug.

DISCOVERY OF METFORMIN

The study behind the evolution of metformin is connected with Galega officinalis, an herbal medicine found in Europe. Further investigation was carried out in 1800s, and it was discovered that it contains a significant amount of guanidine, particularly in the immature seed pods, which has the potential to lower blood sugar levels [5]. Afterwards, the use was stopped because of its toxicity. Later, metformin was reintroduced in 1940s. The French Physician Jean Sterene disclose the use of metformin for the management of diabetes in 1957. During 1920s, several animal studies were conducted with monoguanidine derivatives and found that they lowered blood sugar levels [6]. Metformin consists of two guanidine groups with a loss of ammonia. Preliminary studies found that biguanides diminished oxygen consumption; later, it was found that they reduced mitochondrial oxygen consumption. The important mechanism behind metformin is the abolishment of hepatic gluconeogenesis. Additionally, metformin has crucial effect on cAMP- PKA signaling. It was found that metformin improved lipid metabolism and hepatic mitochondrial functions.

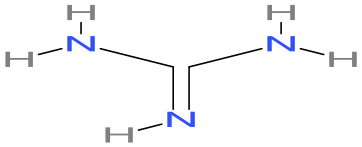

Fig. 1(a)

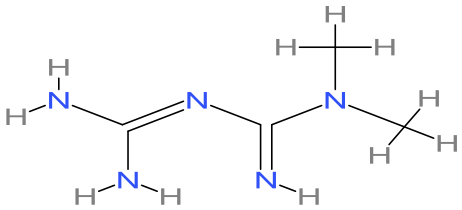

Fig.1 (b)

Fig. 1(a), 1(b) Structure of guanidine and Metformin

Metformin (1, 1- dimethylbiguanide hydrochloride) which is a hydrophilic molecule, available as oral doses of 500 to 1000 mg. It has maximum plasma concentration (2.5 hours) and elimination half-life of 6-7 hours. Metformin can be used as a combination therapy with different regimens. The most popular combination therapies are Metformin with Sulfonyl ureas, Insulins, Thiazolidinediones, DDP-4 inhibitors.

ADVERSE EFFECTS OF METFORMIN

Lactic acidosis: A lethal negative effect is lactic acidosis, which is formed due to anaerobic metabolism i.e. mitochondrial respiration, which causes lactate generation. Serum lactate levels exceeding 5 mmol/L and an arterial pH below 7.35 are defined as metformin-associated lactic acidosis (MALA). Metformin associated lactic acidosis is rare.

GI effects: The most effects are diarrhea, nausea and abdominal discomfort. It may happen due to the collection of drugs in the small intestine. But now extended release Metformin is available which have fewer GI symptoms [7].

Vitamin B 12 Deficiency: Prolonged treatment with Metformin can lead to Cobalamin deficiency. The positive charge of biguanide molecule force out calcium ions; calcium plays a vital role in absorption of Cobalamin. So low calcium will lead to cobalamin deficiency. This Cobalamin deficiency shows neurological damages i.e., peripheral neuropathy.

VITAMIN B 12

Cobalamin, also known as vitamin B 12, is found in red meat, shell fishes, milk, eggs and dairy products. Once consumed in vitamin B12 rich foods, it is freed from carrier proteins through proteolysis. In stomach, it is strapped with haptocorrin. Salivary glands are the production area of haptocorrin. The bonded haptocorrin – vitamin B 12 enters the duodenum; haptocorrin breakdowns in the presence of pancreatic proteases, and vitamin B 12 becomes free. Vitamin B 12 has a crucial role in DNA synthesis and neuroprotection. On the other hand, its deficiency will lead to hematological abnormalities such as megaloblastic anemia and hyper segmented neutrophils [8]. The Vitamin B 12 is stored in liver and some amount in kidneys. Vitamin B12 from diet is normally bound to proteins. The bonded Vitamin B12 is released into the stomach under the effect of gastric acid and pepsin. Liver and kidneys act as the main store house of vitamin B12. A human liver has a capacity to store 1.0 - 1.5 mg of vitamin B12 [9]. Vitamin B12 goes to enterohepatic circulation and excretion through bile along with reabsorption in the ileum. The estimated amount of Vitamin B12 secreted in bile was found to be 4 mcg [10].

VITAMIN B 12 DEFICIENCY

Generally, some cases that leads to deficiency of vitamin B 12 are malnutrition, older age, vegetarian diet, H. pylori infection, autoimmune diseases, prolonged use of antacids, celiac diseases, Crohn's disease, parasitic infections, along with use of some categories of drugs like Cholestyramine, Neomycin and Para amino salicylic acid. In each of the above, there is a known mechanism behind it. For example, vegetarian diet will lead to cobalamin deficiency because cobalamin found to be rich in animal sources. Prolonged use of

proton pump inhibitors declines the production of hydrochloric acid by gastric parietal cells, and as a result, cobalamin is not properly released from foods. Drugs like cholestyramine and antibiotics inhibits the endocytosis of intrinsic factor and vitamin B 12 complex. The uptake of cobalamin slows down in the presence of celiac and crohn's disease. Furthermore, pancreatic disorders will affect the proteolytic degradation of cobalamin [8].

HOW METFORMIN AFFECT VITAMIN B 12

Metformin has the capacity to improve peripheral insulin sensitivity and bring down risk of cardio vascular mortality. Furthermore, it has additional outcomes on vascular protection along with weight loss. Several reports indicates that use of metformin will lead to reduced uptake of vitamin B 12. Biguanide groups have a positive charge, so they will force out divalent cations like calcium. The absorption of vitamin B 12 is calcium dependent and it will interfere with metformin [11]. The exact mechanism behind how metformin induces vitamin B 12 deficiency is not fully understood. Studies forward the following aspects regarding the mechanism: obstruction of calcium-dependent binding of cobalamin with intrinsic factors, interference with cubilin endocytic receptor, variation of intestinal motility, changes in bile acid metabolism and declined intrinsic factor secretion [8]. Metformin induced vitamin B12 deficiency shows neurological damage, but the markers of specific condition is misdiagnosed as diabetic peripheral neuropathy. Early manifestation and management of vitamin B 12 deficiency is paramount in patients with metformin therapy [12]. In elderly patients, vitamin B 12 status should be checked according to the dose of metformin. More studies shows that patients who have been taking metformin for at least 5 years also have a wide risk of vitamin B 12 deficiency [13]. Metformin induced vitamin B 12 deficiency subdivided according to two aspects, i.e., duration of use and dose. Routine checkup of vitamin B 12 is crucial in patients taking metformin because markers of vitamin B 12 deficiency are hard to identify and can lead to irreversible conditions. Routine screening should be done, especially in persons who receive more than 2000 mg of metformin per day, and that too for a longer period of time more than 4 years [11]. Screening of functional indicators such as homocysteine and MMA (Methyl malonic acid) may be helpful to identify an exact vitamin B 12 deficiency [8].

PREVALENCE OF COBALAMINE DEFICIENCY AMONG PATIENTS WITH TYPE 2 DM

Many of the studies reported that prolonged use of Metformin lower the Cobalamin level specifically taken at a dose of 2000 mg/day.

Kim et al. from his cross sectional study established that use of metformin for at least 6 months in a dose range of >1500 mg per day may indicate as a key factor for vitamin B 12 deficiency[13].

De Groot- Kamphius et al. found that patients who have received treatment with metformin had a remarkable prevalence of vitamin B 12 deficiency compared with patients who have not taken metformin [14].

De Jagar et al. reported a powerful proof of Metformin associated low cobalamin level through his 4.3 year duration of randomized controlled trial. The study found that Metformin associated Cobalamin deficiency is 19% [15].

Liu Q et al. confirmed a positive association between Metformin induced Vitamin B12 deficiency through his meta- analysis [16].

Raheel Iftikhar et al. from his case control study found that patients undergoing Metformin therapy, 31% showed Vitamin B 12 deficiency [17].

Table 1: Prevalence of Metformin – Vitamin B12 deficiency, A correlation with clinical studies conducted

Study	Prevalence of Metformin induced Cobalamin deficiency	Mean age of patients	Metformin dose mg/ day	Duration of therapy (years)	Study setting
DeGroot- Kamphuis et al.	14.1%	62.6	NA	4.9	Netherland outpatient clinic
DeJager et al.	9.9%	64	2050	4.3	Netherland outpatient clinic
Raheel Iftikhar et al.	31 %	56	2100	8	Department of outpatient medicine, Kharian military hospital
Ahmed et al.	28.1	58.5	2400	9.6	South Africa Outpatient diabetes clinic
Kim et al.	22.2%	60	2000	6	Yongin Severance Hospital Korea

DIAGNOSIS OF VITAMIN B 12 DEFICIENCY

Total vitamin B_{12} measurement is used as a cost effective parameter, but in people with vitamin B_{12} concentrations <400 pmol/L, it has limited sensitivity and specificity [18]. Homocysteine (Hcy) and Methylmalonic acid (MMA) concentrations increase in cobalamin deficient persons and therefore, it can be considered as a specific markers of cobalamin deficiency [19]. Tests such as total Vitamin B 12, holo-TC-11, both will measure the circulating vitamin but Homocysteine and MMA will measure cobalamin at the cellular level. Hence, more precise biomarkers have their own sensitivity [20]. The following confirmatory testes are used for the diagnosis of cobalamin deficiency:

SERUM VITAMIN B 12 TEST

In general, the serum Vitamin B12 test has a good level of sensitivity for determining vitamin B 12 status. In patients with megaloblastic anemia, vitamin B 12 levels below 148 pmol/L had a sensitivity above 95% [21]. In pregnancy and folate deficiency Vitamin B 12 levels were falsely reported [22]. Elevated cobalamin in serum is the marker of a serious condition such as promyleocytic leukemia, hypereosinophilic syndrome and polycythemia vera [23]. Additionally, people with renal illness had higher Vitamin B 12 amounts [24].

HOLO TC II TEST (HOLOTRANSCOBALAMINE TEST)

The circulating Cobalamin will bound to TC I and TC II carrier proteins up to 80%. The bounded Cobalamin generally an inert complex. Holo- TC refers to the part that is bound to the part that is bound to TC II protein. Metabolic status of Vitamin B 12 is the main determinant of Holo-TC II serum levels moreover, the Holo TC levels will interrupt the absorption of Vitamin B 12[25]. Study reports suggest that amount of Holo TC II will be associated with folate disorder, myelodysplasia, alcoholism etc. [26-28].

HOMOCYSTEINE TEST

Homocysteine test can be used as a test to determine the metabolic status of Vitamin B 12 because high levels of homocysteine is linked to Vitamin B 12 insufficiency. In patients who receive Metformin have elevated homocysteine level compared to non-users of Metformin [29]. De Jager et al., conducted a randomized trial and confirmed significant elevation of homocysteine level after 4 years of metformin therapy. Factors like renal failure, old age, Vitamin B2 and Vitamin B6 will show elevated homocysteine concentration [30].

MMA TEST

Cobalamin undergoes catalysis by the enzyme methylmalonyl – CoA mutase and synthesizes succinyl CoA. These reactions were happened in mitochondria. Therefore, declining cobalamin results in elevated MMA levels [31]. A cross sectional study conducted by Wile DJ et al. reported that Type 2 DM and Metformin therapy showed higher MMA compared to group not taking

Metformin therapy[32]. Amount of MMA can be measured by using gas chromatography- mass spectroscopy with a typical reference range of 0.08-0.56 micro mol/L of methyl malonic acid [33].

MANIFESTATIONS OF COBALAMINE DEFICIENCY

Cobalamin deficiency is a clinically dominant problem. The hematological manifestations include macrocytosis and megaloblastic anemia [34]. Neurological symptoms can occur in different forms, such as peripheral neuropathy, optic neuropathy, mood disorders along with depressive symptoms [35]. Once cobalamin deficiency occurs, it leads to irritable bowel motility i.e. constipation or diarrhea, loss of bladder control, an impaired immune response and low bone density [36-38].

CONSEQUENCES OF METFORMIN INDUCED COBALAMINE DEFICIENCY

Peripheral neuropathy is the major complication of Type 2 DM. The somatic and autonomic components of the nervous system are both affected by diabetic neuropathy, which is a broad set of illnesses with an incredibly complex etiology. The most common form of diabetic neuropathy - distal symmetric polyneuropathy [39]. With the progression of the condition, diabetic neuropathy prevalence also shifts. In fact, after 10 years of follow-up, the prevalence of diabetic neuropathy in T_2 DM patients rise from 8% to 42% [40]. In a large cohort of patients enrolled in the BARI 2D experiment who had more advanced T2 DM and coronary artery disease, 50% had verified diabetic neuropathy at baseline [41]. However, no investigations on the relationship between metformin and low Vitamin B12 have found any significant effects on hematological data like hemoglobin concentrations such as macrocytosis, mean corpuscular volume, anemia [42].

MANAGEMENT OF DIABETIC NEUROPATHY

Control of hyperglycemia: Studies have reported that achieving normoglycemic control will help manage the neuropathies. There is one study conducted by Buolton, Andrew JM et al., suggests that patients with painful neuropathy managed with insulin infusion for four months [43]. Glycemic control was measured by glycated hemoglobin levels and regular checking of blood sugar levels. Another study done by Muir J et al, will compare those who have painful neuropathy with painless neuropathy and suggest that those who have symptoms of pain have poor control [44]. Pharmacological management include several classes of drugs like Tricyclic antidepressants, through prevention of reuptake of norepinephrine and serotonin at the synapse of pain control system [45]. The two medications with the most experience are Amitriptyline and Imipramine. Another class of drugs, Selective Serotonin Reuptake Inhibitors especially Paroxetine and Citalopram, was found to be more efficacious [46]. Another study conducted by Edward KR et al., with Gabapentin, which is an anticonvulsant, showed significant relief from painful neuropathy [47].

MANAGEMENT OF COBALAMIN DEFICIENCY

There are currently no recommendations or guidelines for the supplementation and proper dosage of cobalamin for diabetic patients taking Metformin. Monthly injections of cobalamin or high therapeutic dose almost 1000mcg of cobalamin with calcium were included in the management [48]. The amount of intake of Vitamin B 12 depends on the severity and its underlying cause [49]. The use of multivitamins are convenient, inexpensive but it is not sufficient in patients have treatment with Metformin. A study conducted by Reinstatler et al. suggested that 6 mcg of cobalamin everyday was insufficient [50]. An extended period use of oral cobalamin at a dose of 25mcg per day is important to

maintain normal cobalamin status with 2000 mg of Metformin [51].

CONCLUSION

Numerous experimental studies reported that management of type 2 diabetes mellitus with first line oral hypoglycemic drug Metformin leads to significant deficiency of vitamin B 12 (cobalamin). Metformin is currently used to manage various conditions including prediabetes, PCOS, insulin resistance etc. The possible negative reaction of metformin is due to extended period use and higher dose therapy. Serum vitamin B 12 level is strictly measured in patients who are at higher risk of vitamin B 12 deficiency due to factors such as age, vegetarian diet along with taking metformin. The American Diabetes Association guidelines now suggest that routine evaluation of serum cobalamin should be done in patients receiving metformin. The evaluation is very important because the indicators of vitamin B 12 deficiency can be mistaken for peripheral neuropathy, which will worsen the situation of patients. The cost behind the checking of vitamin B 12 is fairly low. Patients who have cobalamin deficiency, vitamin B 12 supplementation is effective for the prevention of peripheral nerve damage. Physicians should care about the screening of vitamin B 12 in patients under metformin therapy.

Declaration by Authors

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