Variations in Serum Vitamin B12 Levels among Metformin Receiving and Non-Receiving Type 2 Diabetes Mellitus Patients - A Case Control Study in a Tertiary Care Hospital in Puducherry

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ABSTRACT

BACKGROUND

Metformin is considered as a cornerstone in the treatment of diabetes and is the most frequently prescribed first line therapy for individuals with T2DM. Metformin use in type 2 diabetes mellitus has shown to cause vitamin B12 (B12) deficiency. Besides these, B12 deficiency related neuropathy may mimic and/or aggravate the underlying diabetic neuropathy. Furthermore, there have been lack of consensus regarding the universal routine screening of serum B12 levels among metformin users with T2DM, and similar studies from India are also limited. So, we wanted to study the relationship between metformin use and serum B12 in T2DM patients.

METHODS

The study included a total of 104 subjects of which 52 are T2DM affected individuals who are only on metformin therapy for at least 3 months, served as cases and 52 are newly diagnosed T2DM age and sex matched individuals yet to be medically treated, served as controls. Their blood samples were collected to estimate vitamin B12 and other parameters.

RESULTS

The median B12 levels in cases 213.5 pg/mL (IQR 136.25-301.0) were found to be significantly lower ($P < 0.05$) when compared to median levels of controls 240 pg/mL (IQR 214-303.25). B12 levels also showed a significant fall in their levels with increasing duration of T2DM.

CONCLUSIONS

Metformin induced vitamin B12 deficiency plays a major role in developing life risks in T2DM patients. Routine screening and supplementation of B12 may be required in T2DM patients

KEYWORDS

Metformin, Type 2 Diabetes Mellitus, Vitamin B12

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BACKGROUND

Type 2 diabetes mellitus (T2DM) has reached global pandemic proportion in the last 2 decades and is the fifth leading cause of death worldwide. Hyperglycemia the major sequel of T2DM, is associated with micro and macro vascular complications which imposes tremendous burden on the individuals as well as on the entire health care system.[1] In the global burden of diabetes, presently 415 million are affected, which is expected to rise about 642 million in 2040 and almost 193 million people in the world are still undiagnosed for T2DM. In India it is still more alarming, having 69.2 million T2DM affected people in 2015 which is expected to rise about 123.5 million in 2040.[2] Among the countries, India stands second to China in the prevalence of diabetes.[3] T2DM is a group of metabolic disorders characterized by hyperglycemia resulting from variable degrees of insulin resistance and impaired insulin secretion, leading to a state of relative insulin deficiency.[4] It was formerly known as non-insulin dependent diabetes mellitus (NIDDM) or adult-onset diabetes. This is in contrast to T1DM, which was formerly known as insulin dependent diabetes mellitus (IDDM) or juvenile diabetes.[5] This classification based on age or insulin-dependence is obsolete because, many T2DM patients require exogenous insulin for glycemic control, and about 5 – 10 % of T1DM is seen in adults greater than 30 years of age and also nowadays T2DM is more frequently diagnosed among children and adolescents who are obese. In view of that, the current system of DM classification by American Diabetes Association (ADA) uses the terms T1DM and T2DM, along with the other specific types and GDM.[6] Pre-diabetes include impaired fasting glucose (IFG) and impaired glucose tolerance (IGT),they are at a higher risk to develop diabetes in the near future.[7] Long duration of hyperglycemia in diabetes is associated with long-term damage, dysfunction and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.[8]

The main pathogenesis behind the development of T2DM are insulin resistance (IR), and β–cell dysfunction. Of this, IR often proceeds the insulin secretory defect by more than a decade. This explains the body's compensatory mechanism that enables the person to remain asymptomatic for a long time before diagnosed as T2DM. IR is the decreased ability of the peripheral cells (particularly, skeletal muscles, liver and fat) to respond to the actions of insulin.[4] It is the characteristic feature of T2DM. IR is commonly detected 10 - 20 years before the onset of T2DM which makes it a good predictor for the future progression of the disease to T2DM. Both genetic and environmental risk factors act synergistically to produce IR.

The β-cells try to compensate by over working after the development of IR, leading to a state of hyperinsulinemia. Later the progression leads to the poor performance of β-cells, which leads to insulin deficiency. This failure is termed as the β-cells dysfunction causing IR in T2DM.[9] The majority of the people suffering from T2DM are obese, with central visceral adiposity. Therefore, the adipose tissue plays an important role in the pathogenesis of T2DM.[10]

When the blood glucose levels are more than 180 mg/dL then hyperglycemia will lead to glycosuria. Since glucose is osmotically active it leads to polyuria. Polyuria leads to dehydration and increase the thirst reflex which causes polydipsia. T2DM is characterized by a generalized catabolic state, resulting in a paradoxical coupling of polyphagia with weight loss and muscle wasting. The triad polyuria, polydipsia and polyphagia, along with weight loss are the classical features of T2DM.[11]

According to ADA, the criteria for diagnosing diabetes mellitus are, either a fasting plasma glucose (FBG) ≥ 126 mg/dL or a post prandial glucose (2-PG) ≥ 200 mg/dL during OGTT (75-gm) or a glycated hemoglobin A1c levels of ≥ 6.5% or a random plasma glucose (RPG) ≥ 200 mg/dL or individuals with symptoms of hyperglycemia or hyperglycemic crisis[12]

Other tests apart from FBG, 2- PG (OGTT), RPG and A1C, include test for spot urine albumin: creatinine ratio, urine microalbumin, lipid profile, self-monitoring of blood glucose, lipid profile, serum creatinine (to estimate GFR), ketone bodies in blood, arterial blood gas (ABG), serum electrolytes, plasma osmolality. Peripheral vessel Doppler studies, electrocardiogram (ECG), blood pressure (BP), foot examination and ophthalmic fundal examination tests are done.[13]

Acute complications of T2DM are diabetic keto acidosis (DKA). DKA is more common in both T1DM and T2DM. The minute amount of insulin secreted can prevent the hepatic ketoacidosis in T2DM. It results in elevated ketone bodies in blood and in urine which is called ketonemia and ketonuria which together known as ketosis. Hyperkalemia and hyponatremia are seen as a result of electrolyte disturbances. Blood sugar is in the range of 250 - 600 mg/dL. Fruity odour breath is a characteristic feature which is caused by Acetone. Nausea, vomiting, abdominal pain, hypotension, dehydration, lethargy and coma are the other signs and symptoms of DKA.[14] Hypoglycemia due to over dosage of exogenous insulin. Lactic acidosis due to T2DM or due to side-effects of metformin. Hyperosmolar hyperglycemic state (HSS) is also due to non-compliance to insulin or oral hypoglycemic agents (OHAs), causing a severe hyperglycemia of about 600 - 1200 mg/dL. This results in severe dehydration and hyper osmosis. There is minimal metabolic acidosis and no ketosis and electrolytes are normal.[15]

The high degree of morbidity and mortality associated with T2DM are because of its chronic complications. In due course of time almost all the diabetics are prone to develop complications. Poor glycemic control and advanced age are the risk factors. Multicenter trials like diabetes control and complications trial (DCCT), UK prospective diabetes study(UKPDS) and the Kumamoto study insists the early diagnosis and strict glycemic control using intensive therapeutic regimes, to delay the disease progression and to prevent the complications.[16] Micro-vascular complications include diabetic retinopathy, diabetic nephropathy and diabetic neuropathy whereas macro-vascular complications include coronary artery disease, peripheral vascular disease, hypertension, stroke and lower extremity gangrene.[17]

Metformin (1,1-dimethylbiguanide), a biguanide derivative is one of the most generally used oral hypoglycemic agents in conjugation with life style modification (diet, weight control and physical activity).[18] It has been used for more than 50 years and was approved by the US Food and Drug Administration (FDA) in 1994. Metformin is now the most widely used anti-diabetic drug, with almost all guidelines throughout the world recommending metformin as the first-line treatment for patients with T2DM. It typically reduces the basal and post-prandial hyperglycemia by approximately 25 % in more than 90 % of patients when given alone (or) with other therapies. Metformin may also be used to treat other conditions involving insulin resistance, such as poly cystic ovary syndrome (PCOS). Metformin also has beneficial effects on carbohydrate metabolism, weight loss, and vascular protection, with fewer important side effects.[19]

Metformin is the drug of choice for patients with T2DM. It is recommended as the first line oral therapy for T2DM in the recent guidelines of the American diabetes association and European association of the study of diabetes. In case of overweight diabetic patients, the risk of death is decreased due to intensive glucose control with metformin.[20]

Metformin being prescribed to at least 120 million people worldwide. It lowers glucose concentrations without causing overt hypoglycemia in T2DM. Metformin reduces insulin resistance and fasting plasma insulin level and hence called as "Insulin Sensitizer".[21] Three different studies have previously examined the effect of metformin on the rates of net hepatic glycogenolysis and gluconeogenesis in patients with T2DM. Stumvoll et al. in their study found that metformin decreases endogenous glucose production through 37 % reduction in rates of synthesis of glucose from non-carbohydrate sources.[22] Primary function of metformin is to reduce hepatic glucose production by inhibiting gluconeogenesis.[23] The preferential action of metformin is predominant expression of the OCT1 which facilitates cellular uptake of metformin.[24] Metformin activates AMP activated protein kinase (AMPK) which protects cellular functions under energy restricted conditions. Mitochondria is the primary target of the drug which induces mild and specific inhibition in the mitochondrial respiratory–chain complex I.[25] The positive charge and a polar hydrocarbon side chain of the drug promotes its binding to the mitochondria. The drug also blocks the reverse electron flow through the respiratory chain-complex I and inhibits mitochondrial production of ROS.[26]

Vitamin B12 or cobalamin is a vital nutrient for health. It plays an important role in the functioning of the brain and nervous system, DNA synthesis and in the formation of red blood cells. The principle source of vitamin B12 are animal foods including meat and dairy products, as well as foods fortified with the vitamin.[27] A serum value of less than 150 pg/mL is considered to be of definite deficient, a value less than 220 pg/mL is considered deficient and a value of more than 220 pg/mL is considered normal. The recommended dietary allowances (RDA) for adults is 4- 5 µg/day in adults, 6 µg/day in pregnancy and 8 µg/day in lactation. The total storage is about 2 to 5 mg in adult man. Mainly stored in liver and to a lesser extent in kidneys.[28] Metabolism of vitamin B12 involves its release from animal sources which is mediated by gastric acid and pepsin. B12 binds to R protein secreted by salivary glands. R-protein is hydrolyzed by alkaline pancreatic proteases in the duodenum. Then finally B12 binds with the intrinsic factor secreted by gastric parietal cells. The B12-IF complex is resistant to proteolytic degradation. On the terminal ileum the complex attaches to the specific receptors which is calcium mediated. The complex degrades and the vitamin is released. The free vitamin attaches to transcobalamin II and released to circulation. This complex is also called as holotranscobalamin and it is utilized by liver, bone marrow and other vital organs. Up to 90% of body's total vitamin is stored in liver.[29]

If any one of the steps is disrupted it will result in clinical or biochemical vitamin B 12 deficiency. Factors include smoking, alcoholics, vegetarian diet and malabsorption due to celiac disease, chronic pancreatitis and pernicious anemia.[30] Various physiological role are performed by vitamin B12. It mediates the methylation process of homocysteine to methionine and the conversion of methylmalonyl coA to succinyl coA. It acts as a cofactor for the transfer for methyl group for myelin, neurotransmitters and membrane phospholipids.

It is required for conversion of dietary folate into its active metabolic form tetrahydrofolate.[31] It is essential for proper functioning of nervous system, also for the synthesis of monoamines or neurotransmitters like serotonin and dopamine. It is very important for the normal hemopoiesis.[32]

Despite its positive glycemic lowering effect, metformin has long been associated with lowering vitamin B12 levels. Patients on long-term metformin therapy were found to be at the risk of anemia. This may be due to a metformin related vitamin B12 reduction. The prevalence of B12 deficiency among T2DM patients on metformin therapy ranges from 5.8 to 33 %.[33]

Furthermore, there have been lack of consensus regarding the universal routine screening of serum B12 levels among metformin users with T2DM, we planned a case control study comparing vitamin B12 levels in T2DM subjects with and without the exposure of metformin.

Aim

To study the relationship between metformin use and serum B12 levels in subjects with T2DM.

Objectives

1. To estimate the serum B12 level in subjects with T2DM on metformin therapy and to compare that with age and gender matched T2DM subjects not on metformin therapy.
2. To correlate the serum B12 levels with duration of diabetes and dosage of metformin.

METHODS

We performed a case control study which was carried out from January 2016 to December 2016 in subjects with T2DM

diagnosed using ADA criteria who reported to Pondicherry Institute of Medical Sciences, Pondicherry.

Inclusion Criteria

This study involved 104 T2DM subjects aged between 30 and 60 years, out of which 52 cases were on metformin treatment and 52 controls were newly diagnosed, yet to be treated. A detailed history of duration of diabetes type of oral hypoglycemic drug and dosage of drug was collected and verified from the patients. Their dietary history was also enquired.

Exclusion Criteria

Pregnant women, alcoholics, vegans, smokers, people on B12 supplements, individuals with liver diseases or malabsorption all were excluded from the study.

The serum samples obtained from the subjects were analyzed for vitamin B12 which is not done routinely for all patients, by electrochemiluminescence assay (ECLIA) in Cobas e411 auto analyzer. On the basis of their B12 levels, subjects were divided into normal (> 220 pg/mL), possible deficiency (150 - 220 pg/mL) and definite deficiency (< 150 pg/mL). The blood samples were collected from the patients an overnight fasting (no calorie intake for at least 8 hours), centrifuged immediately and the serum was used for the following analysis fasting blood glucose (FBG) and vitamin B12. The required sample size 104 (each group 52) was calculated based on the mean difference of serum B12 is 53.5 pg/ml, power of 80 % and level of significance 5 %. This is a self-funded study was approved by the Institutions Ethics Committee. The confidentiality of the study reports has been maintained strictly and all the queries and apprehensions of the patients and their families were addressed with utmost care and a written informed consent was taken.

Statistical Analysis

Data was entered in MS Excel 2007 and analyzed by statistical package for social sciences (SPSS) 20.0 version. The collected data was summarized with numbers and percentages for categorical variables and mean and standards deviation or median and interquartile range were used for quantitative variables. The study population was divided into different groups based on sex (male, female), age group (30 - 60 years) and dosage of metformin treatment and duration of diabetes mellitus. The differential distribution of FBS and vitamin B12 levels amongst these groups were analyzed using Mann-Whitney U test. For correlating vitamin B12 levels with duration of diabetes mellitus the Spearman's rank correlation test was used. P - value < 0.05 was considered as statistically significant.

RESULTS

The study was done on 104 individuals with T2DM. It included 52 (25 males and 27 females) cases of T2DM who are only on metformin therapy and 52 (25 males and 27 females) newly diagnosed subjects yet to be treated. The mean age limit is 47 years. The proportion of patients with possible vitamin deficiency were higher in cases than in controls (Fig 1).

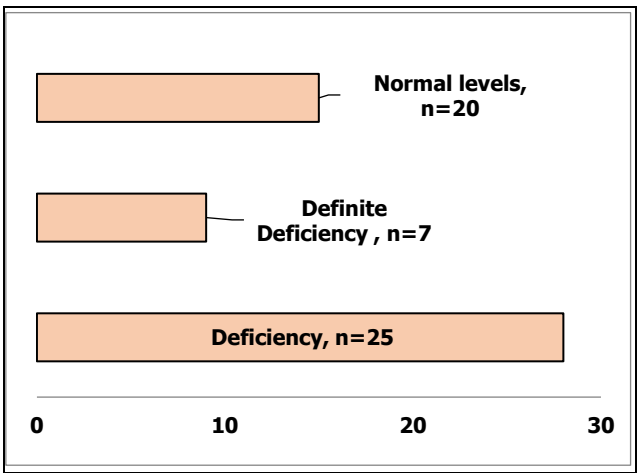

Figure 1. Vitamin B12 Distribution among Cases

	Controls n=52 Median (IQR)	Cases n=52 Median (IQR)	P - Value
Vitamin B12 (pg/ml)	240 (214 - 303.25)	213.5 (136.25 - 301.0)	0.025*

Table 1. Comparison of Vitamin B12 Levels in Controls and Cases by Mann-Whitney U Test

P value < 0.05, statistically significant

Serum vitamin B12 levels were significantly higher in non-metformin group as compared with metformin group (Table 1). We also found a significant difference in the fasting blood sugar values of cases and controls. There existed a significant negative correlation between the duration of metformin intake and serum vitamin B12 levels (Fig. 2 & Table 2).

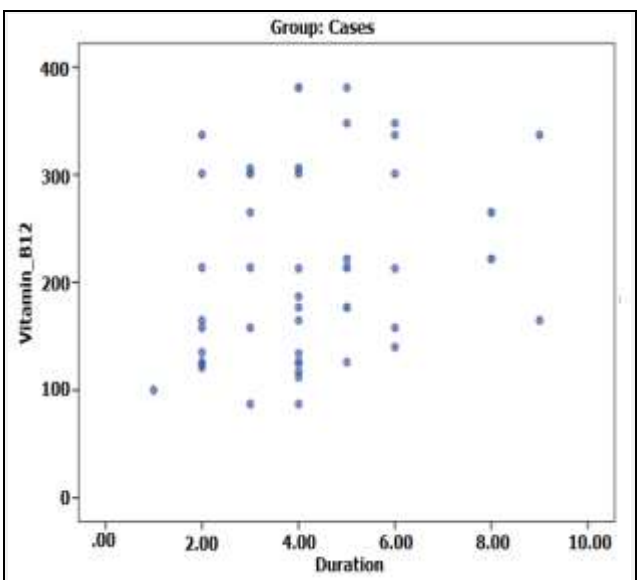

Figure 2. Correlation between Vitamin B12 Levels and Duration of Diabetes Mellitus

Vitamin B12 VS Duration of Diabetes	
r -0.287	P 0.039*

Table 2. Correlation between Vitamin B12 and Duration of Diabetes Mellitus by Spearman's Correlation Test

*p<0.05 statistically significant

We categorized the study subjects as normal (B12 > 220 pg/mL) and deficient (B12 < 220 pg/mL) among the cases and controls to find the Odds ratio.

Vitamin B12 Status	Cases n (%)	Controls n (%)	Total	OR	P
Deficient	30 (63.80 %)	17 (36.20 %)	47	2.80 (1.26 - 6.24)	0.01*
Normal	22 (38.60 %)	35 (61.40 %)	57		
Total	52	52	104		

Table 3. Odds Ratio Calculation

*P < 0.05 statistically significant

DISCUSSION

Our study showed significant decrease in vitamin B12 level in T2DM patients those who are only on metformin therapy. In metformin exposed group of 52 subjects, 25 had B12 deficiency (< 220 pg/ml) and 7 had definite vitamin B12 deficiency (< 150 pg/ml) (Fig 1). This has important implications because India has a high burden of uncontrolled T2DM and vegetarian population who are at high risk of vitamin B12 deficiency. We found that metformin exposed group had lower vitamin B 12 when compared with people with non-metformin exposure (Table1). We found out a statistically significant Odds ratio of 2.8, strongly emphasizing that, metformin treatment was associated with a decrease in serum vitamin B12 levels (Table 3). In a study done by Singh et al. after estimating the vitamin B12 levels in T2DM subjects exposed to metformin, they demonstrated a significant association between metformin use and vitamin B12 deficiency and they also found that the severity of peripheral neuropathy increases with cumulative metformin dose and the duration of the therapy.[34]

The proposed mechanisms to explain metformin induced vitamin B12 deficiency among patients with T2DM include: alterations in small bowel motility which stimulates bacterial overgrowth and consequential vitamin B12 deficiency, competitive inhibition or inactivation of vitamin B12 absorption, alterations in IF levels and interaction with cubulin endocytic receptor.[35] vitamin B12 forms a complex with cubulin (endocytic) receptor at ileum for absorption. The complex is normally taken by ileal cell surface by calcium dependent process. Metformin with protonated biguanide group binds to the B12 - cubulin complex and imparts positive charge to it, alters membrane potential and competitively repels the divalence calcium ions thus preventing calcium dependent uptake, leading to malabsorption of B12.[36] This inhibitory effect is reversed with calcium supplementation.[37] The side effects of metformin has been demonstrated in several ensuing cross sectional studies.[38] Metformin - induced B12 malabsorption may be due to digestive changes, which leads to the binding of B12 - intrinsic factor (IF) complex and a reduction of B12 absorption.[39] Since the B12 - IF complex binds to the ileal cell surface receptor, metformin alterations in IF levels and/ or ileal morphological structure may lead to B12 reduction.[40]

In our study we found a statistically significant negative correlation between the duration of diabetes and the B12 levels (Fig 2 & Table 2). Kos' cohort study found, vitamin B12 levels of T2DM patients treated with metformin for more than 4 years were significantly lower than control.[41] Greibe's cohort study demonstrated that, when compared to placebo metformin decreased serum vitamin B12 in PCOS women after 6 months of treatment.[42] In a study conducted in Japan, metformin is associated with B12 deficiency along with hyperhomocysteinemia, there by increasing the cardiovascular complications. They also found that the patient with micro-vascular complications like retinopathy had still lower levels of B12 compared to DM patient without complications and the incidence of these complications is high in metformin taking group compared to other oral hypoglycemic agents.[43] Cognitive impairment and the progression of diabetic peripheral neuropathy may also be accelerated by metformin in a Vitamin B12 dependent manner.[44] Kibrige et al. in their study, emphasizes the routine supplementation of vitamin B12 supplementation irrespective of their B12 status because it may prevent the risk of dementia, degeneration of spinal cord and impaired memory etc.[30]

CONCLUSIONS

Vitamin B12 is an important and essential micronutrient for normal functioning of life. B12 deficiency is highly prevalent among patients with T2DM on metformin therapy. There are no proper guidelines advocating routine screening for B12 deficiency among T2DM patients on metformin therapy. Screening for vitamin B12 deficiency and appropriate supplementation among diabetic patients may help in reducing the long-term complications associated with both diabetes and B12 deficiency.

Limitations

In our study we aimed only to study the association between the metformin intake and changes in serum B12 levels in cases and controls. Though we have eliminated the effect of confounders like age and sex by matching the cases and controls, we couldn't make out the effect of other confounders using regression analysis with the available data.

Data sharing statement provided by the authors is available with the full text of this article at jebmh.com.
Financial or other competing interests: None.
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