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Prevalence of Vitamin B 12 Deficiency and its Association with Metformin-Treated Type 2 Diabetic Patients in a Tertiary Care Hospital

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Prevalence of Vitamin B 12 Deficiency and its Association with Metformin-Treated Type 2 Diabetic Patients in a Tertiary Care Hospital

Background: Type 2 diabetes mellitus is a heterogeneous group of disorders characterized by variable degree of insulin resistance, impaired insulin secretion and increased glucose production. Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. One of the efficient antidiabetic drugs forming a milestone in the treatment of T2DM is Metformin.

Subjects and Methods: Total 56 out of which 28 T2DM patients of the age group 36-65 of both sex who were on Metformin, duration of treatment for a minimum of seven months and dosage of metformin of at least 1000mg/day were included in the study along with 28 healthy controls.

Results: Significant increased levels of FBS, HbA1c, insulin and C-peptide in the study group. It was observed that the mean levels of vitamin B12 markers homocysteine and methylmalonic acid were significantly higher and low level ofvitamin B12 was found in patients who were on metformin treatment.

Conclusion: Vitamin B12 insufficiency was frequently found in my population and may progress into B12 deficiency.

Keywords: Type 2 DM, Vitamin B12, Metformin

INTRODUCTION

One of the efficient anti-diabetic drugs forming a milestone in the treatment of T2DM is Metformin. This biguanide is prescribed clinically for the past 60 years as a first line oral anti-diabetic agent. Every year Metformin caters to the need of more than 150 million people, the reason being its therapeutic efficacy and affordable price.[1]

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T2DM is a heterogeneous group of disorders characterized by variable degree of insulin resistance, impaired insulin secretion and increased glucose production. Diabetes is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease.[2] The mechanism of action of Metformin in improving hyperglycemia involves in improving the signaling of insulin together with suppressing hepatic gluconeogenesis. Vitamin B12 deficiency results as one of the few side effects of metformin usage. This is being overlooked and rarely investigated owing to the numerous clinical benefits of metformin.[2,3]

Even well-educated health providers overlook such an easily diagnosed and inexpensively treated condition which leads to a frustrating B12 deficiency epidemic. Untreated B12 deficiency can cause hypercellular and dysplastic bone marrow. This can be mistaken for signs of acute leukaemia. It is to be noted here that B12 levels are normal 50% of the patients with subclinical disease.[4] Vitamin B12 plays a vital role in DNA and cellular metabolism by serving as an essential cofactor in methylation process. Hence a deficiency leads to DNA disruption and derangement of cellular metabolism. This might lead to serious clinical consequences. Vitamin B12 is intracellularly converted to two active coenzymes, adenosylcobalamin in mitochondria and methylcobalamin in the cytoplasm. They are necessary for the homeostasis of Methyl malonic acid (MMA) and Homocysteine (HC). Methylmalonic acid is converted intosuccinyl-CoA, of which vitamin B12 is a cofactor for the reaction. Homocysteine is biosynthesized from methionine then resynthesized into methionine or converted into amino acidcysteine.[5]

The markers which are increased early in vitamin B12 deficiency include serum methyl malonic acid and homocysteine. Measurement of serum methyl malonic acid and homocysteine levels are more sensitive methods of screening for vitamin B12 deficiency.[6] High levels of methyl malonic acid and homocysteine have been identified as better indicators of B-12 deficiency than the actual serum B-12 level itself. A deficiency of vitamin B-12at the tissue level elevates the levels of both MMA and HC even when serum vitamin B-12 concentrations are within the reference values. Elevated MMA and HC levels together have been found to be 99.8% sensitive for diagnosing functional vitamin B-12 deficiency.[7] Aim of the present study was to association of vitamin B12 status in patients with type 2 diabetes mellitus on metformin.

SUBJECTS AND METHODS

Present study was conducted in Department of Medicine, Venkateshwara Institute of Medical Sciences, Gajraula, India. Cases included the T2DM patients on metformin treatment. The patients were selected as per the following criteria. Total 56 out of which 28 T2DM patients of the age group 36-65 of both sex who were on Metformin, duration of treatment for a minimum of seven months and dosage of metformin of at least 1000mg/day were included in the study along with 28 healthy controls. Following were excluded from the study; Type 2 Diabetic Patients with any co existing cause for B12 deficiency, Pregnant ladies and Type 2 Diabetic Patients on other drugs and Malabsorption syndromes

Biochemical Analysis

After an overnight fast of about 10-12 hours, 10 ml of venous blood was collected from each participant. The samples were allowed to retract and then spun at 2500 rpm for 5 minutes to obtain serum samples which were kept at -200C until analysed. Samples were analyzed for the following study variables vitamin B12, homocysteine, methyl malonic acid, plasma insulin and CPeptide using chemiluminescence (ROCHE Cobas e 411). HbA1C was estimated by Nephlometric method.

Statistical Analysis

Statistical analysis was done by IBM (SPSS 22) version. ShapiroWilk test was performed to see whether the continuous variables follow normal Gaussian distribution. To compare vitamin B12 and glycemic status, student ‘t’ test was performed and to find the correlation between vitamin B12 deficiency markers with glycemic status in cases, Pearson correlation analysis was performed. P value less than 0.05 was considered as significant.

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Table 1: Comparison of biochemical marker between cases and controls:

Parameters	T2DM	Controls	P value
Glycated Hemoglobin (%)	8.9±3.6	4.6±0.7	0.02
Fating Blood Glucose (mg/dl)	159.2±28.56	96.4±14.9	0.01
Insulin	8.62±3.4	6.2±2.1	0.001
Homocysteine	13.4±4.6	6.4±2.5	0.01
Methyl Malonic Acid	155.6±34.6	121±29.7	0.01
C-Peptide	4.3±0.6	2.5±0.7	0.01
Vitamin B12	218.2±56.1	374.5±67.4	0.001

Table 2: Association of Glycemic Status with vitaminB12 deficiency markers:

Biochemical Parameters	HC(r)	P value	MMA (r)	P value
HbA1c	0.44	0.01	0.42	0.02
C-peptide	0.36	0.05	0.38	0.04
Fasting Blood Glucose	0.38	0.04	0.42	0.02
Insulin	0.42	0.02	0.36	0.05

RESULTS AND DISCUSSION

Table-1 shows biochemical markers of the patients with type 2 diabetes. The youngest patient was aged 36 years while the oldest patient was aged 65 years. Mean age of the study group was 46.7±9.2 years, while it was 42.6±7.23 in control group, which was statistically non-significant.52% patients in study group and 56% patients in controls were male. In the study group BMI ranged from 24 to 30kg/m2 with a mean of 26.4±6.4 kg/m2. In the control group BMI ranged from 20 to 28kg/m2 with a mean of 24.6±6.3 kg/m2. Significantly higher mean was observed in mean BMI in study group as compared to controls (p=0.02). Results showed significant increased levels of FBS, HbA1c, insulin and Cpeptide in the study group. It was observed that the mean levels of vitamin B12 markers homocysteine and methylmalonic acid were significantly higher and low level of vitamin B12 was found in patients who were on metformin treatment Table-1. We have correlated the markers of glycemic status with the markers of Vitamin B12 deficiency Homocysteine and Methylmalonic acid. The results showed positive correlation between the two markers was depicted in Table-2. Reports have shown that metformin use have a significant impact on the concentration of vitamin B12 in patients with T2DM.8 The percentages of patients with vitamin B12 deficiency and with borderline deficiency observed in this study are in line with the report of Nervo et al.9 This observation could be an indication of nutritional deficiency or a consequence of metformin use. Mean BMI of

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patients in study group was 26.4±6.4 kg/m2. BMI ranged from 24 to 30kg/m2. In the control group BMI ranged from 20 to 28kg/m2 with a mean of 24.6±6.3 kg/m2. Significantly higher mean was observed in mean BMI in study group as compared to controls (p=0.02). Similar results were observed by studies done in the past. A large proportion of Asian and Chinese patients with type 2 diabetes are of normal weight: a recently published pooled cross-sectional analysis of 39,794 diabetes patients from Asia (most of whom had type 2 diabetes), revealed that 64% had a BMI < 25 kg/m2. One regional Chinese study showed that 59.2% of 521 diabetes patients in Hong Kong had a BMI < 25 kg/m2 and another study showed that 36% of 4,160 patients in Shanghai had a BMI < 24 kg/m2.[10]

Total serum vitamin B12 is a relatively insensitive and unspecific biomarker of deficiency that does not reflect recent variations in cobalamin status. Holotranscobalamin (holoTC), the metabolically active portion of vitamin B12, is the earliest laboratory parameter that becomes decreased in case of a vitamin B12 negative balance. Concentration of methylmalonic acid (MMA) is a functional vitamin B12 marker that will increase when the vitamin B12 stores are depleted. Isolated lowering of holoTC shows vitamin B12 depletion (negative balance), while lowered holoTC plus elevated MMA (and homocysteine) indicates a metabolically manifested vitamin B12 deficiency, although there still may be no clinical symptoms.[11]

The concentration of total homocysteine (tHcy) in serum and plasma is elevated in both folate and cobalamin deficiencies, whereas methylmalonic acid (MMA) in serum, plasma, or urine is a specific marker of cobalamin function. The combined measurement of both metabolites is useful for the diagnosis and follow-up of these deficiency states. In addition, total homocysteine is elevated under various pathologic states (eg, renal failure), and hyperhomocysteinemia is associated with an increased risk of cardiovascular disease, cognitive dysfunction, and adverse pregnancy outcomes.[12]

The relationship between insulin and homocysteine is controversial. However, handful of studies have found strong associations between insulin and homocysteine. One study in humans found that B-12 and Folate therapy resulted in a number of improved parameters among patients with metabolic syndrome.[13] HbA1c is an independent risk factor for type 2 diabetes. Subjects with high-normal levels of HbA1c deserve particular attention because they have a strong risk of developing diabetes.[14]

In this study the correlation between fasting blood glucose and HbA1c was analyzed with the markers of vitamin B12 and no correlation existed between them. In the transition from normal glucose tolerance (NGT) to type 2 diabetes mellitus (T2DM), the role of β-cell dysfunction and peripheral insulin resistance (IR) is well established. In this study there is a significant correlation between insulin and C-peptide with the markers of vitamin B12 status. The mechanism of metformin inhibition of absorption is not fully understood. The current theory of inhibition is based on interference with intrinsic factor binding in the gut. It would be assumed that an increase in concentration of the inhibiting substance (metformin) would result in an increase in inhibition of said absorption. As metformin is excreted unchanged in urine with an elimination half-life of approximately 5 hours (Graham et al) it would be logical for the relationship between metformin and vitamin B12 malabsorption to be somewhat linear.[15]

CONCLUSION

These findings suggest that, vitamin B12 insufficiency was frequently found in my population and may progress into B12 deficiency. There is moderate correlation between the markers of B12 status and levels of fasting blood sugar as well as HbA1C. There is a significant correlation between insulin and C-peptide with the markers of vitamin B12 status.

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