VOLUME-7, ISSUE-11, NOVEMBER-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper

Biochemistry

"ESTIMATION OF VITAMIN B12 LEVELS IN PATIENTS WITH TYPE 2 DIABETES MELLITUS ON METFORMIN: A HOSPITAL BASED STUDY"

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ABSTRACT

BACKGROUND: Metformin is considered as the first-line antidiabetic agent in the treatment of type 2 diabetes mellitus (T2DM) due to its effect on glucose and lipid metabolism. The mechanism of action of Metformin in improving hyperglycemia involves in improving the signaling of insulin together with suppressing hepatic gluconeogenesis.

METHODS: 45 patients with type-2 diabetic patients on metformin therapy with an age ranged from 30 to 65 years along with 45 healthy controls were recruited from the General Medicine Department of HMCH, Rourkela according to inclusion and exclusion criteria.

RESULTS: The mean age and BMI of both the cases and controls were 48.62 ± 7.94 and 43.52 ± 5.24; 28.04 ± 4.34 and 26.54 ± 3.32 respectively. Mean serum vitamin B12 levels in the case group was 217.45±86.32 and in control was 370.08±91.6and the difference was statistically significant.

CONCLUSION: Metformin which is first line oral hypoglycemic agent as recommended by ADA is significantly associated with decrease in vitamin B12. 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.

KEYWORDS : Vitamin B12, Fasting Blood Glucose and Type 2 DM.

INTRODUCTION:

Metformin is considered as the first-line antidiabetic agent in the treatment of type 2 diabetes mellitus (T2DM) due to its effect on glucose and lipid metabolism.[1] Every year Metformin caters to the need of more than 150 million people, the reason being its therapeutic efficacy and affordable price.[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.[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, also called cobalamin, is a water-soluble vitamin involved in the optimal functioning of the hemopoetic, neuro-cognitive and vascular systems. It is involved in DNA synthesis, fatty acid metabolism and energy production.[5] 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 twoactive 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.[6] Markers which are increased early in vitamin B12 deficiency include serum MMA and HC. Measurement of serum MMA and HC levels are more sensitive methods of screening for vitamin B12 deficiency.[7] High levels of MMA and HC 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.[7] This study was therefore, carried out to evaluate the serum levels of vitamin B12 in patients with T2DM on metformin.

MATERIAL AND METHODS:

This study was conducted in the Department of Biochemistry in association with Department of Medicine, Hi-Tech Medical College & Hospital Rourkela, Odisha, India, during the period from November 2016 to October 2017. Randomly selected, 45 patients with type-2 diabetic patients on metformin therapy with an age ranged from 30 to 65 years along with 45 healthy controls were recruited from the General Medicine Department of HMCH, Rourkela according to inclusion and exclusion criteria. After an overnight fast of about 10-12 hours, 5 ml of venous blood was collected from each participant. The samples were allowed to retract and then spun at 3000 rpm for 12 minutes to obtain serum samples which were kept at -20^{0}c until analysed for vitamin B12 level. Fasting blood glucose by GOD POD and HbA1C was estimated by cation exchange resin methods. Samples were analyzed for the following study variables vitamin B12, homocysteine, methyl malonic acid, plasma insulin and C-Peptide using Fully automated Cobas e 411. Statistical analysis of data was performed using the SPSS (Version 16.0). For comparison of parameters between the two groups, students t test was used. Statistical significance was considered at a 'p' value of < 0.05. For correlation, Pearson's correlation coefficient (r) was used.

RESULTS AND DISCUSSION:

The base line characteristics of the case and control group are shown in Table I. The mean age and BMI of both the cases and controls were 48.62 ± 7.94 and 43.52 ± 5.24; 28.04 ± 4.34 and 26.54 ± 3.32 respectively. Mean serum vitamin B12 levels in the case group was 217.45±86.32 and in control was 370.08±91.6and the difference was statistically significant. This present study (Table-1) showed the 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 of vitamin B12 was found in patients who were on metformin therapy. Reports have shown that metformin use have a significant impact on the concentration of vitamin B12 in patients with T2DM.[8,9,10] We have correlated the markers of glycemic status with the markers of Vitamin B12 deficiency Homocysteine and Methylmalonic acid. Table 2 showed the positive correlation between these two markers. Total serum vitamin B12 is a relatively insensitive and unspecific biomarker of deficiency that does not reflect recent variations in cobalamin status. Holotranscobalamin, the metabolically active portion of vitamin B12, is the earliest laboratory parameter that becomes decreased in case of a vitamin

B12 negative balance.

Table 1: Compare of glycemic and vitamin b12 status with cases and controls:

Biochemical markers	Case 45 Mean±SD	Control 45 Mean±SD
Age in yrs	48.62 ± 7.94	43.52 ± 5.24*
Height (m)	1.62 ± 0.08	1.60 ± 0.06*
Body weight (kg)	72.34 ± 11.56	66.24 ± 9.91*
BMI (kg/m2)	28.04 ± 4.34	26.54 ± 3.32*
Fasting glucose	151.02±63.25	78.63±16.05
HbA1c %	8.32±3.04	4.41±0.7
Fasting insulin	8.32±4.26	6.21±3.34
Homocysteine	13.21±4.8	7.26±2.76
C-peptide	4.25±1.02	2.6±0.82
Methyl Malonic acid	156.41±78.26	123±85.21
Vitamin B12	217.45±86.32	370.08±91.6

(Statistically Significant at p value <0.05) *NS: Statistically not Significant

Table 2: Correlation of glycemic status with vitamin B12deficiency markers:

Parameters	Homocysteine (r)	P-value	Methylmalonic Acid (r)	P-value
FBS	+0.45	0.001	+0.32	0.03
HbA1c	+0.37	0.01	+0.36	0.01
Insulin	+0.42	0.01	+0.43	0.003
C-peptide	+0.48	0.000	+0.46	0.001

(Statistically Significant at p value <0.05)

Concentration of methylmalonic acid is a functional vitamin B12 marker that will increase when the vitamin B12 stores are depleted. Isolated lowering of holotranscobalamin shows vitamin B12 depletion (negative balance), while lowered holotranscobalamin plus elevated methylmalonic acid (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 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 Glycated hemoglobin(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 to type 2 diabetes mellitus, the role of β-cell dysfunction and peripheral insulin resistance is well established.In this study there is a significant correlation between insulin and C-peptide with the markers of vitamin B12 status.

CONCLUSION:

These findings suggest that metformin which is first line oral hypoglycemic agent as recommended by ADA is significantly associated with decrease in vitamin B12. 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. So we recommend that vitamin B12 should be measured prior to initiation of metformin therapy and later annually in patients of type2 diabetes mellitus who are on metformin therapy

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