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EFFECT OF GLYCEMIC CONTROL ON VITAMIN B12 METABOLISM IN TYPE 2 DIABETES MELLITUS PATIENTS

[1]Dhiraj Mahaseth, [2]Vikas Kumar Gupta, [3]Bijay Kumar Mahaseth, [4]Ashish Kumar Sharma

[1]Assistant Professor, Madhubani Medical College and Hospital, Madhubani, Bihar, India
[2]Associate Professor Maharshi Vashishtha Autonomous State Medical College, Basti, U.P.
[3]Assistant Professor, N.C. Medical College & hospital, Israna, Panipat, Harayana, India
[4]Assistant Professor, Madhav Prasad Tripathi Medical College, Siddharthnagar, U.P. India

Corresponding Author
Ashish Kumar Sharma
Assistant Professor,
Madhav Prasad Tripathi Medical College, Siddharthnagar, U.P. India
ashishgrmcg@gmail.com

Abstract

Background: Diabetes mellitus is a metabolic disorder due to absolute or relative insulin deficiency. Polyuria in type 2 DM leads to loss of important water soluble nutrients in urine. In view of widespread deficiency of vitamin B12 in our country and increasing prevalence of diabetes mellitus type 2, it is considered worthwhile to assess vitamin B12 status of patients with diabetes mellitus type 2.

Material and methods: Present study comprises of 60 subjects of both sexes whom 30 are healthy controls and 30 are clinically confirmed cases of diabetes mellitus, age group ranges from 40-70 yrs. Serum separated from plain vial after centrifugation was used for estimation of serum vitamin B12 by ELISA, Plasma glucose estimation by GOD-POD Method, End Point, Glycated hemoglobin by Boronate Affinity chromatography (NycoCard).

Result: In our study it was found that serum vitamin B12 (218.24 ± 68.31 pg/ml) was significantly lower in type2 diabetes mellitus patients compared to serum vitamin B12 (254.20 ± 64.89 pg/ml) of controls. Serum vitamin B12 level (190.20 ± 19.44 pg/ml) is significantly lower in diabetes mellitus patients who were suffering from the disease for more than 3.5 years as compared to serum vitamin B12 level (257.18 ± 88.74 pg/ml) of diabetes mellitus patients who were suffering from the disease for less than 3.5 years.

Conclusion: Thus, it can be concluded that there is significant vitamin B12 deficiency in type 2 diabetes mellitus patients.

Key words: Diabetes, Vitamin B12, Metformin, Duration of diabetes

Introduction: Diabetes mellitus is a metabolic disorder due to absolute or relative insulin deficiency [1, 2]. It is a chronic disease that causes serious health complications including kidney failure, heart disease, stroke, diabetic retinopathy and diabetic neuropathy [3]. Polyuria in type 2 diabetes mellitus (T2DM) patients may leads to the loss of important water soluble nutrients in urine [4]. Since, the neurological manifestations of vitamin B12 deficiency coincide with the neurological manifestation in T2DM [5, 6]; it has been proposed that vitamin B12 deficiency at least in part may be responsible for such neurological manifestations in these patients.

In India, a country with a large proportion of vegetarians due to cultural and religious beliefs, very high prevalence of vitamin B12 deficiency among the general population has been reported in many studies [7-9]. In view of widespread deficiency of vitamin B12 in our country and increasing prevalence of diabetes mellitus type 2, it is considered worthwhile to assess vitamin B12 status of patients with diabetes mellitus type 2. Hence, this study is intended to determine the effect of glycemic control on vitamin B12 metabolism in type 2 diabetes mellitus patients and compare it with that of controls.

Material and Methods:

Subject selection:- Present study comprises of 60 subjects of both sexes whom 30 are healthy controls and 30 are clinically confirmed cases of diabetes mellitus, age group ranges from 40-70 years. Informed consent was taken from each control and case before collecting the blood sample. The patients taking any medications which are likely to affect vitamin B12 metabolism, patients with renal failure, malignancies, pregnant and lactating women have been excluded from this study.

Sample collection:-

After overnight fasting for 8 hours, 5 ml of venous blood was drawn with aseptic precautions from antecubital vein of all the subjects.

Analysis of Sample: Serum separated from plain vial after centrifugation was used for estimation of serum vitamin B12 by ELISA, Plasma glucose estimation by GOD-POD Method, End Point, Glycated hemoglobin by Boronate Affinity chromatography (NycoCard).

Statistical analysis:-Mean ± SD were calculated for all the parameters analysed and were compared by Student's t-test using SPSS. P-values considered significant were as follows:-

$P < 0.05$ – As Significant, $P < 0.001$ – As Highly significant

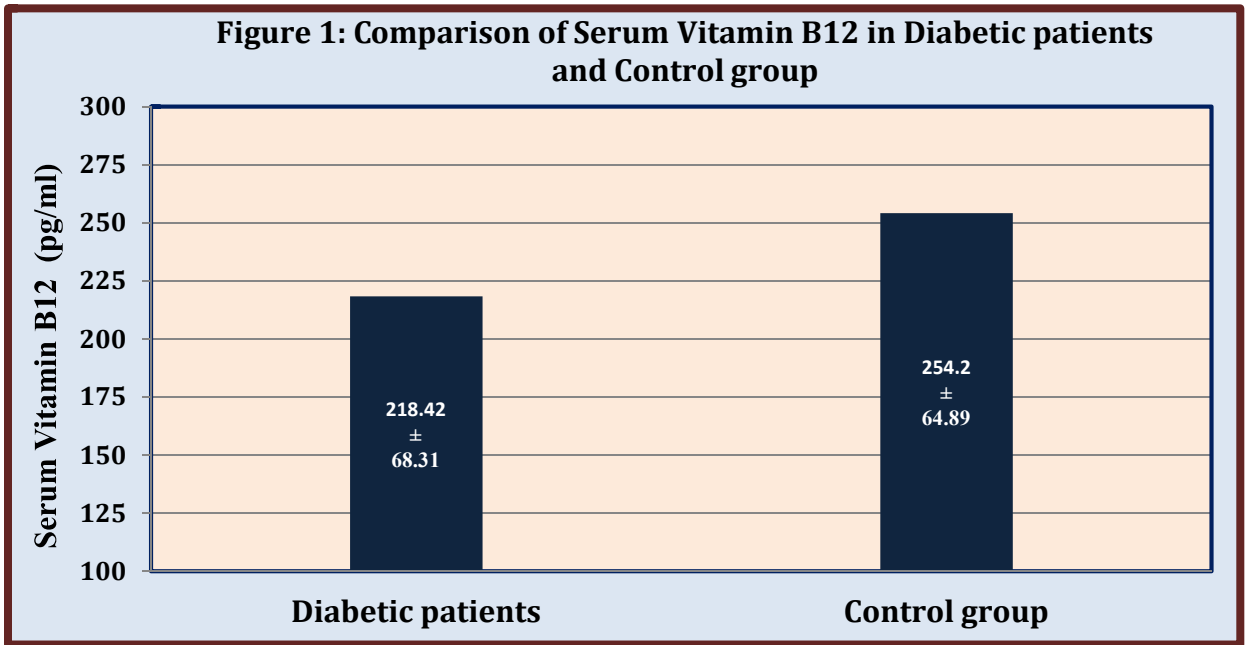
Observation and results: Table and figure 1 showing the difference in the mean level of serum vitamin B12 (pg/ml) in diabetics (218.24 ± 68.31) and in controls (254.20 ± 64.89) was found to be statistically significant. Table and figure 2 showing the Vitamin B12 level is significantly lower in diabetes mellitus patients who were suffering from the disease for more than 3.5 years.

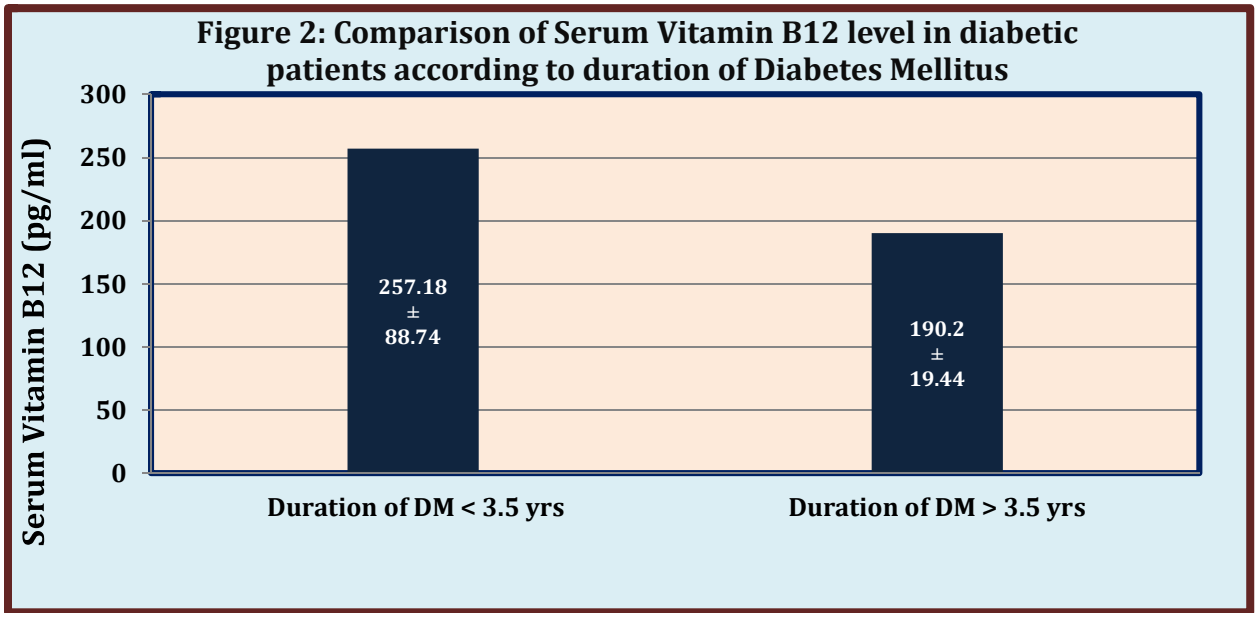
Table 1: Mean and Standard deviation of measured parameters in diabetic and non diabetic patients of Serum Vitamin B12

S.N.	Parameters	Diabetic group (Mean ± SD)	Control group (Mean ± SD)	t-value		p value
				Diabetic group	Control group	
1.	Vitamin B12 (pg/ml)	218.24 ± 68.31	254.20 ± 64.89	17.51	21.24	0.00

Table2. Mean and Standard deviation of parameters in diabetic patients according to the duration of diabetes mellitus

S.N.	Parameters	Duration of DM < 3.5 yrs (Mean ± SD)	Duration of DM > 3.5 yrs (Mean ± SD)	t- value	p- value
1.	Vitamin B12 (pg/ml)	257.18 ± 88.74	190.20 ± 19.44	3.03	0.005





Discussion: The present study was undertaken to assess the serum parameters of vitamin B12 metabolism in type 2 diabetes mellitus patients and compare with that of controls. A cross sectional study consisting of 60 subjects out of which 30 patients having type 2 diabetes mellitus and 30 normal healthy controls were selected. In our study it was found that serum vitamin B12 (218.24 ± 68.31 pg/ml) was significantly lower in type2 diabetes mellitus patients compared to serum vitamin B12 (254.20 ± 64.89 pg/ml) of controls. Serum vitamin B12 level (190.20 ± 19.44 pg/ml) is significantly lower in diabetes mellitus patients who were suffering from the disease for more than 3.5 years as compared to serum vitamin B12 level (257.18 ± 88.74 pg/ml) of diabetes mellitus patients who were suffering from the disease for less than 3.5 years.

According to our findings, serum vitamin B12 was lower in patients with type 2 diabetes because majority of patients with type 2 diabetes were using metformin as oral hypoglycaemic drug. It has been reported earlier that, metformin causes vitamin B12 deficiency as a side effect [10-12]. This condition may lead to vitamin B12 deficiency in type 2 diabetes mellitus patients. Deficiency of vitamin B12 in humans is associated with neuropathy, megaloblastic anaemia and often irreversible neurological disorders [13].

Ebesunun et al in 2012 found that there was significantly low level of serum vitamin B12 (48.47 ± 0.74 µg/L) in type 2 diabetes mellitus patients as compared to vitamin B12 level (58.48 ± 1.51µg/L) in normal healthy controls [14]. In another study by Fahmy E et al in 2012 were also found that there was significantly low level of serum vitamin B12 (393.73 ± 181.61 pmol /L) in type 2 diabetes mellitus as compared to serum vitamin B12 level (950.73 ± 428.72 pmol /L) in normal healthy controls [15]. The above studies done by previous researchers were consistent with our findings that serum vitamin B12 level was decreased in type 2 diabetes mellitus patients.

Marar O et al found that there were significantly lower serum vitamin B12 levels in type 2 diabetes mellitus patients on metformin as compared to type 2 diabetes mellitus without metformin [16]. Another study done by Alachkar A et al have shown that there was significant decrease in serum vitamin B12 concentration (524.5 ± 105 vs. 470 ± 140 pg/ml, P=0.0057) in type 2 diabetes mellitus patients on metformin therapy for 6 months [17]. Niafar M et al have shown that the type 2 diabetes mellitus patients receiving metformin had significantly lower serum vitamin B12 levels (320.94±141.34 vs 408.50±175.07 pmol/l, P<0.001) and higher prevalence of vitamin B12 deficiency [18]. In a nested case control study performed among 155 adult Chinese diabetes mellitus patients on metformin and 310 controls, every 1 g/day increase in the metformin dose conferred more vitamin B12 deficiency. Among patients using metformin for more than 3 years were more vitamin B12 deficient as compared with those who had received metformin for less than 3 years [19].

The risk of developing metformin associated vitamin B12 deficiency is greatly influenced by increasing age, metformin dose and duration of use. These studies have shown that long term treatment of metformin increases the chances vitamin B12 deficiency manifold, which is similar to the findings obtained from this study [20].The mechanism of vitamin B12 deficiency with metformin is due to malabsorption of vitamin B12 at its absorption site in the terminal ileum. Initially, it was believed that metformin caused proliferation of bacteria in the small bowel either due to an effect on intestinal motility or an increased intestinal glucose level.

Diabetes mellitus patients may have slow intestinal transit causing bacterial overgrowth and vitamin B12 malabsorption [21]. However, the current and more likely explanation for metformin-induced vitamin B12 malabsorption and deficiency is that metformin has an effect on calcium-dependent membrane action in the terminal ileum. Absorption of the vitamin B12-intrinsic factor complex is calcium dependent process and metformin interferes with this absorption. Some research evidence suggests that dietary calcium supplementation reverses metformin-induced vitamin B-12 malabsorption. Intrinsic factor (IF), also produced by gastric parietal cells, is required for vitamin B12 absorption from the GI tract.

Metformin treatment can result in vitamin B12 deficiency mediated by depression of IF secretion. The risk of adverse effects from metformin induced vitamin B-12 malabsorption will increase with the time of exposure to metformin. In experimental studies suggests that after partial gastrectomy and removal of intrinsic factor; it takes twelve to fifteen years for serum

vitamin B-12 levels to become deficient. Therefore in patients who have been on long-term metformin treatment was vitamin B-12 deficient [22, 23]

A recently published follow up study from the United States of America showed that administration of oral vitamin B12 among type 2 diabetes mellitus patients on long term use of metformin was ineffective in correcting biochemical vitamin B12 deficiency [24]. One of the consequences of vitamin B12 deficiency is increased homocysteine because homocysteine cannot be converted into methionine in vitamin B12 deficiency. Increased homocysteine causes oxidative stress which worsens the diabetic condition and may leads to many cardiovascular diseases. Long term use of metformin may also be responsible for diabetic nephropathy and neuropathy mainly because of vitamin B12 deficiency which is required for proper development of neurons. Many type 2 diabetes mellitus patients with vitamin B12 deficiency due to metformin have no or only mild anemia and macrocytosis [25].

The limitation of our study was that we did not measure methylmalonic acid levels which can improve the sensitivity of results by identifying B12 deficiency in an early asymptomatic stage. Serum concentration of homocysteine as well as serum and urinary concentrations of methylmalonic acid are elevated in cobalamin deficiency, due to a decreased rate of metabolism [26].

Conclusion: Thus, it can be concluded that there is significant vitamin B12 deficiency in type 2 diabetes mellitus patients. Higher vitamin B12 deficiency occurs in type 2 diabetes mellitus patients taking metformin for long period of time. Oxidative stress induced nephropathy and osmotic diuresis seen in these patients may also be additional factors responsible for the loss of vitamin B12 in urine. Further studies are suggested to evaluate the effect of vitamin B12 replacement in these patients towards reducing vitamin B12 deficiency and associated symptoms.

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