

RESEARCH ARTICLE

Comparative Observational Study of Serum Vitamin B12 Levels in Newly Diagnosed Type 2 Diabetes Mellitus and Non-diabetic Patients

Nahid Rao¹, Pallavi Godiyal¹, Vaishali Sati¹, Nitin Bansal¹, Reenoo Jauhari²

ABSTRACT

Background: Methylcobalamin or vitamin B12 is a coenzyme involved in the synthesis of methionine, pyrimidine and purine bases. Type 2 diabetes is associated with oxidative stress; vitamin B12 deficiency in diabetic patients taking metformin has been linked with oxidative stress. Because of these associations, it is implied that vitamin B12 deficiency must be considered a risk factor for complications related to diabetes. However, several studies also suggest that deficiency of vitamin B12 starts from the early stages of diabetes. It has also been suggested that vitamin B12 supplementation may help in reducing the complications related to diabetes. Although, the use of prophylactic Vitamin B12 to reduce complications related to diabetes remains controversial.

Objective: This study aimed to explore the clinical relevance of vitamin B12 in newly diagnosed type 2 diabetes mellitus (T2DM) patients. This study measured vitamin B12 levels in newly diagnosed patients with T2DM and explored the correlations between its serum levels and various metabolic parameters in T2DM.

Materials and methods: This was a one year, single-centre, prospective, comparative, observational study. To collect data on age, smoking history, alcohol consumption, physical activity status, family history of T2DM and medications, all subjects completed a self-answered questionnaire. Plasma samples were used to assay the biochemical parameters such as fasting blood sugar (FBS), post prandial blood sugar (PPBS), Glycated Hemoglobin (HbA1c), lipid profile and serum Vitamin B12. The participants were divided into newly diagnosed T2DM patients (n = 300) and healthy control subjects (n = 300).

Results: Serum Vitamin B12 were significantly lower in newly diagnosed T2DM patients than in healthy control subjects (362.07 ± 182.25 vs 559.23 ± 275.05 pg/mL; $P < 0.001$). Serum Vitamin B12 correlated negatively with FBS, PPBS and HbA1c in newly diagnosed T2DM patients.

Conclusion: Serum Vitamin B12 levels are significantly decreased in newly diagnosed T2DM patients. Our results suggest that adding Vitamin B12 as a supplement in the early stages of T2DM may contribute to into possible microvascular (neuropathy, retinopathy, nephropathy, etc.) complications related to Type 2 Diabetes Mellitus.

Keywords: Newly diagnosed Diabetes Mellitus, Vitamin B12, Glycemic, Lipid profile, Complications, Progression, Dyslipidemia, Association, Age, Fasting blood sugar.

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INTRODUCTION

Diabetes Mellitus is probably one of the oldest diseases known to human beings.¹ As per the WHO, Diabetes Mellitus (DM) is defined as a heterogeneous metabolic disorder characterised by the common feature of chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism.² Type 2 DM was first described as part of the metabolic syndrome in 1988. The origin and aetiology of DM can vary greatly but always include impairment in insulin production or response or both at some point during the disease. Some patients with diabetes have type 1 diabetes (i.e. immune-mediated or idiopathic). Type 2 DM (also known as non-insulin dependent DM) is the most common type of DM characterized by hyperglycemia, resistance to insulin, and related insulin deficiency. Type 2 DM is a result of interactions between genetic, environmental and behavioural hazards and can also be related to the gestational hormonal environment, genetic defects, other infections, and certain drugs.¹ Methylcobalamin or Vitamin B12 is a coenzyme in the single-carbon metabolic pathways,

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involved in the synthesis of methionine, pyrimidine and purine bases.³ Type 2 diabetes is associated with oxidative stress; Vitamin B12 and folic acid deficiency in diabetic studies have been linked to being with oxidative stress, resulting in hyperhomocysteinemia.⁴ Because of these associations, it

Table 1: Statistical findings of demographic parameters

Factors	Parameters/Variables	Groups		Total	χ^2 value	p-value
		Non-Diabetic(n=300)	Diabetic(n=300)			
Gender	Male	137(45.7%)	138(46.0%)	275	1.016	0.602
	Female	163(54.3%)	162(54.0%)	325		
	Total	300 (100.0%)	300 (100.0%)	600 (100.0%)		
Smoking status(%)	Current	83(27.7%)	93(31.0%)	176 (29.3%)	0.826	0.662
	Former	49 (16.3%)	48 (16.0%)	97(16.2%)		
	Never	168(56.0%)	159(53.0%)	327 (54.5%)		
	Total	300 (100.0%)	300 (100.0%)	600 (100.0%)		
Drinking status(%)	Current	66 (22.0%)	90 (30.0%)	156 (26.0%)	5.800	0.055
	Former	58 (19.3%)	60 (20.0%)	118 (19.7%)		
	Never	176 (58.7%)	150 (50.0%)	326 (54.3%)		
	Total	300 (100.0%)	300 (100.0%)	600(100.0%)		
Exercise status(%)	Moderate	169 (56.3%)	185 (61.7%)	354 (59.0%)	2.771	0.250
	Regular	90 (30.0%)	72 (24.0%)	162 (27.0%)		
	Never	41 (13.7%)	43 (14.3%)	84 (14.0%)		
	Total	300 (100.0%)	300 (100.0%)	600 (100.0%)		

is implied that Vitamin B12 deficiency must be considered a risk factor for complications related to diabetes which most commonly includes peripheral neuropathy, and its development has been linked to hyper-homocysteinemia.⁵

Several studies have reported low levels of Vitamin B12 in plasma in patients with type 2 diabetes who used metformin, this association was further reviewed in a retrospective study of medical records.⁶ But deficiency of Vitamin B12 has also been reported in diabetic patients who do not take metformin. Therefore, it has been suggested that Vitamin B12 supplementation may help in reducing complications and improving the cognitive functions of these individuals.⁷ Although, the use of prophylactic Vitamin B12 to reduce complications related to diabetes remains controversial. Atherosclerosis is another frequent complication related to diabetes.⁸ Diabetes mellitus is the most common cause of fat metabolism disorder which most commonly results in hypertriglyceridemia due to an increase in triglyceride-containing lipoproteins, chylomicrons and very-low-density proteins⁹ Hence, it can be concluded that diabetic patients are more prone to develop hyperlipidemia. Therefore, early preventive actions must be taken to reduce major complications.¹⁰

This study aimed to explore the clinical relevance of Vitamin B12 in newly diagnosed T2DM patients. We measured the serum Vitamin B12 levels of control subjects and T2DM patients and analysed its association with metabolic parameters.

Materials and Methods

Study Design and Patient Population

This was a 1 year, single-centre, prospective, comparative, observational study conducted at Shri Mahant Indires Hospital, Dehradun, India between January 2021 and December 2021. The study was initiated after the approval from the Institutional Ethical Committee. The participants were divided into newly diagnosed T2DM patients ($n = 300$) and healthy control subjects ($n = 300$). The demographic, clinical, and other investigation details were obtained from the patients. The baseline demographic details including age, sex, smoking status, drinking status, physical activity

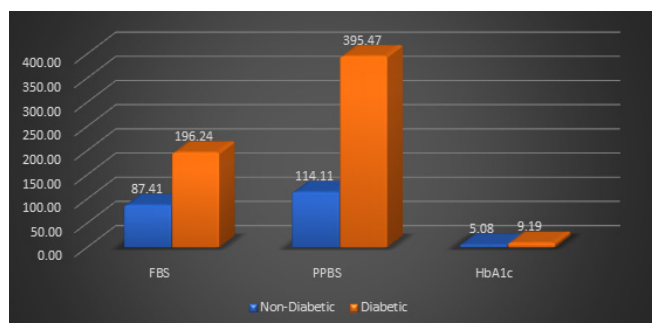


Figure 1: Comparison of FPG, PPG, and HbA1c between Diabetic and non-Diabetic subjects. FPG: fasting plasma glucose; PPG: postprandial glucose, HbA1c glycated haemoglobin

Table 2: Statistical findings of blood sugar and lipid levels

Parameters	Groups	Mean ± Std. Deviation	Mean Difference	t-test value	p-value
AGE (years)	Non-Diabetic	50.43± 9.59	-1.14	-1.492	0.136
	Diabetic	51.57 ± 9.12			
FBS (mg/dl)	Non-Diabetic	87.41±9.49	-108.82	-37.371	0.001*
	Diabetic	196.24± 49.53			
PPBS (mg/dl)	Non-Diabetic	114.11± 11.83	-281.36	-60.467	0.001*
	Diabetic	395.47 ±79.72			
HbA1c (%)	Non-Diabetic	5.08 ± 0.54	-4.11	-16.161	0.001*
	Diabetic	9.19 ±4.37			
HDL (mg/dl)	Non-Diabetic	36.72± 14.98	-1.62	-1.384	0.167
	Diabetic	38.34± 13.64			
LDL (mg/dl)	Non-Diabetic	100.58 ± 41.92	-7.51	-2.091	0.037*
	Diabetic	108.09± 45.94			
Triglycerides (mg/dl)	Non-Diabetic	148.61± 88.90	-34.53	-3.498	0.001*
	Diabetic	183.15± 146.10			
Total Serum cholesterol (mg/dl)	Non-Diabetic	155.58± 53.62	-12.28	-2.714	0.007*
	Diabetic	167.86±57.11			
Vitamin B12 (pg/ml)	Non-Diabetic	559.23± 275.05	197.17	10.350	0.001*
	Diabetic	362.07± 182.25			

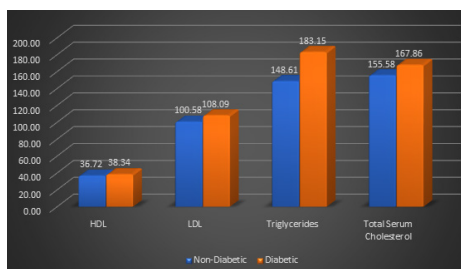


Figure 2: Comparison of HDL, LDL, Triglycerides, and total serum Cholesterol between Diabetic and non-Diabetic subjects. HDL: High-density lipoprotein, LDL: Low-density lipoprotein.

status and biochemical parameters were recorded. The inclusion criteria for the T2DM group were the following: (1) to be diagnosed with T2DM following the guidelines of the WHO; Fasting blood sugar (FBS) ≥ 126 mg/dL or Glycated haemoglobin (HbA1c) $\geq 6.5\%$ or Postprandial blood sugar (PPBS) ≥ 140 mg/dL, (2) to have no diabetic complications and (3) to never have received any diabetes therapy, including medication and insulin. The exclusion criteria for both groups were the following: (1) acute infections, (2) chronic, hepatic and/or renal dysfunction, (3) nutritional disorders, (4) anaemic Patients, (5) pregnant women or (6) any other severe medical illness.

Data/sample Collection and Biochemical Analysis

Self- structured questionnaire was used for the collection of demographic data such as age, gender, smoking history,

alcohol consumption, physical activity status, medical history, family history of T2DM and medications. Plasma samples were used to assay the biochemical parameters. HbA1c was measured by automated CE- HPLC, and FBS and Pure were evaluated by GOD-POD dry chemistry. As per laboratory data reference range, Vitamin B12 was diagnosed as normal (239-931 pg/ml), deficient (<200 pg/ml) by a biochemical test- competitive immunoassay. Lipid profile analysis was performed on the serum obtained from each participant to evaluate total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and triglycerides (TG). Total cholesterol (TC) was determined using CHOD-POD dry chemistry. High-density lipoprotein cholesterol (HDL) and Triglycerides (TG) were measured by enzymatic dry HDL (dry chemistry) and enzymatic endpoint (dry chemistry) respectively. Low-density lipoprotein cholesterol (LDL) was calculated the Friedewald's formula:

$$[\text{LDL Chol}] = [\text{Tot Chol}] - [\text{HDL Chol}] - [\text{TG}]/2.2(1) \text{ mmol/L.}$$

Statistics

Data presentation and statistical analysis were completed using Microsoft Excel 2013, and the Statistical Package for Social Science (SPSS) version 23.0. Data were represented as mean \pm standard deviation (SD). The Chi-square test was used to analyse categorized variables. A p -value <0.005 was considered statistically significant for all analyses. Correlations of Vitamin B12 with glycemic status were analyzed by Pearson correlation.

Table 3: Correlation of Vitamin B12 and glycemic status in the study population

Vitamin B12		Over-all	Non-Diabetic	Diabetic
		Vitamin B12	Vitamin B12	Vitamin B12
Fasting Blood Sugar	Pearson Correlation	-0.419	0.089	-0.228
	p-value	0.001*	0.603	0.045*
Post-prandial Blood sugar	Pearson Correlation	-0.521	0.127	-0.310
	p-value	0.001*	0.410	0.039*
HbA1c	Pearson Correlation	-0.567	0.071	-0.409
	p-value	0.001*	0.495	0.029*

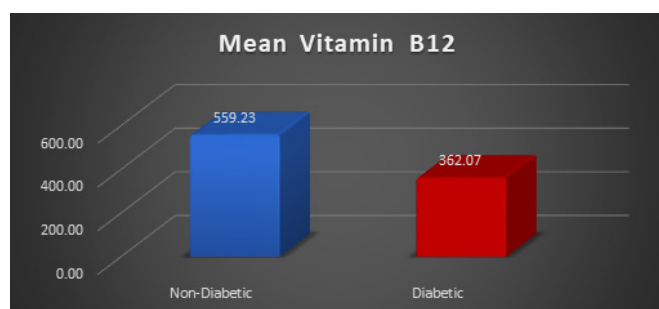


Figure 3: Comparison of Vitamin B12 between Diabetic and non-Diabetic subjects.

RESULTS

A total of 300 participants were included in each group. Based on the results presented in Table 1, there was significant variation in the distribution of gender smoking status, exercise status, and drinking status in both groups

Table 2 shows there was no significant difference in the mean Age between Control and Diabetic subjects. Biochemical parameters such as FBS, PPBS, HbA1c, LDL, TGL and Total serum cholesterol were significantly more among the newly diagnosed Diabetic subjects as compared to control subjects ($p < 0.005$). Although, there was no significant variation in the mean levels of HDL in both the groups. Nonetheless, the mean Vitamin B12 was significantly more among the control subjects as compared to Diabetic subjects.

In addition, the correlation between Vitamin B12 levels with FBS, PPBS, and HbA1c in newly diagnosed was analyzed using Pearson correlation (Table 3, Figure 4). There is a negative correlation of serum Vitamin B12 level with FBS ($r = -0.228$), PPBS ($r = -0.310$) and HbA1c ($r = -0.409$). This means that a decrease in Vitamin B12 is associated with an increase in FBS, PPBS and HbA1c.

DISCUSSION

A study aimed to evaluate the effect of glycemic control on Vitamin B12 status in type 2 diabetes mellitus by Madhura TK et al. divided the subjects into 5 age groups. Only 8% of the patients were in the age group 30-39 years. The maximum

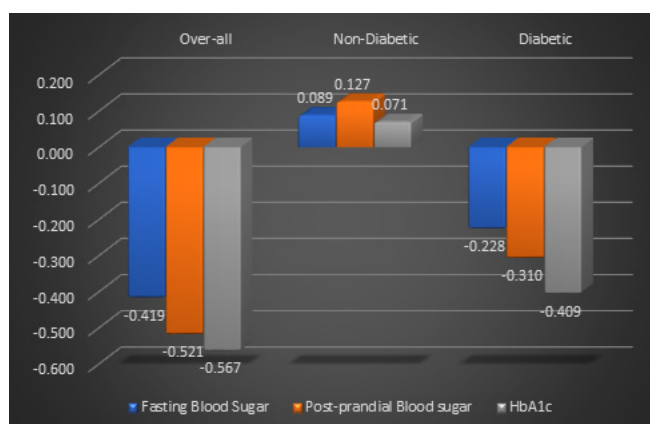


Figure 4: Correlation of Vitamin B12 and glycemic status in the study population

number of patients 31% were in the age group 50-59 years. The mean age of the patients was 54.47 years. The prevalence of T2DM is maximum in the age group 50-59 years. As the duration of the disease progresses HbA1c levels increased and serum Vitamin B12 levels decreased.¹¹ Our study also correlates with the same data. In our study, we observed the mean age of non-diabetic volunteers to be 50.43 ± 9.59 and that of diabetic patients to be 51.72 ± 9.12 . A maximum number of patients were in the age group of 50-60 years in the newly diagnosed diabetic group.

Smokers are 30 to 40% more likely to be at risk of developing diabetes than non-smokers.¹² The evidence from studies of individuals with diabetes shows the strong influence of smoking on mortality^{13-17,18} Although our studies show that 31% of current smokers and 16% of former smokers develop diabetes out of 300 participants, the results may vary in the wider population.

Lower fasting insulin levels and increased insulin sensitivity have been reported in population studies from England,¹⁹ Italy²⁰ and France²¹ as well as in selected groups of individuals such as young adults²² and postmenopausal women.²³ Current or former drinkers had higher glycemic levels when compared to individuals who never drank alcohol.

Physical activity appears to have an independent, beneficial effect on insulin action, glycemic control, and metabolic abnormalities associated with type 2 diabetes.^{24,25} Our research shows that 61.7% of participants were moderate exercisers who have developed diabetes but their HbA1c was lower than the participants who were in the regular activity or never exercising group. Patients diagnosed with type 2 diabetes mellitus had increased Fasting blood sugar (196.24 ± 49.53), postprandial (395.47 ± 7.72) and HbA1c (9.19 ± 4.37) when compared to healthy glucose tolerance subjects (controls).

A study was to determine the recent trend of OCPs residue in blood samples and their association with the known risk factors responsible for developing the risk of diabetes among the North Indian population.²⁶ When the NGT group was

compared with the prediabetes group (Group I vs. Group II), BMI, FBS, and PPBS were found significantly higher ($P=0.025$, $P=0.05$, and $P<0.001$) in the prediabetes group. BMI ($P<0.001$), HbA1c ($P<0.001$), glucose F and PP ($P<0.001$) were found highly significant in the newly detected DM group as compared to NGT (Group 1 vs. Group 3).²⁶

In a study, a total of 110 newly diagnosed T2DM patients and 55 healthy subjects participated. Among the 110 patients with T2DM, 118 (75%) were diagnosed with metabolic syndrome. The mean values for Serum Vitamin B12 in group 1 (control), group 2 (non-MS-associated T2DM) and group 3 (Metabolic syndrome-associated T2DM) of newly diagnosed diabetic patients were recorded to be 633.30 ± 298.44 (pg/ml), 621.16 ± 265.15 (pg/ml) and 506.08 ± 249.57 (pg/ml). In comparison with control patients, the non-MS-associated T2DM patients and patients with MS-associated T2DM had significantly lower levels of Vitamin B12 ($p<0.05$).²⁷

Another study included 165 patients (99 women and 66 men; aged 36–82 years) with newly diagnosed type 2 diabetes mellitus. Baseline serum values of serum B12 in patients with diabetes before treatment with Group 1 and Group 2 were $306.0\text{F}116.8$ and $319.3\text{F}149.6$ (pmol/l) respectively.²⁸

The serum level of B12 was significantly lower in patients (253.3 ± 25.7 pg/ml) compared with controls (688.3 ± 40.9 pg/ml, $t = 63.68$, $P < 0.05$). The low status of folate and B12 is a potential trigger contributing to the development of hyperhomocysteinemia and oxidative stress in patients with T2DM. [4]. Our study shows a negative correlation between Vitamin B12 with the glycaemic status of diabetic patients. And the correlation was statistically significant in terms of p -value (FBS: 0.045^* , PPBS: 0.039^* , HbA1c: 0.029^*) and Pearson correlation (FBS: -0.228 , PPBS: -0.310 , HbA1c: -0.409). The mean value of non-diabetic individuals was found to be: 362.07 ± 182.25 pg/ml and for diabetic, the mean value was: 559.23 ± 275.05 pg/ml. This shows that the patients were on the lower limits of the laboratory reference range.

Diabetics have higher lipid levels than non-diabetics and this abnormality is exaggerated in patients with poor diabetic control.¹⁰ The majority of hyperlipidemic diabetic patients (82.43%) had uncontrolled diabetes i.e. raised fasting blood sugar levels and HbA1C.¹⁰

Few large-scale, real-world studies have assessed the relative associations of lipid fractions with diabetic microvascular events.²⁹ Among subjects with newly diagnosed DM, the mean total cholesterol level was 224.1 mg/dl. The mean triglyceride level was 186.3 mg/dl. The mean HDL cholesterol level was 47.9 mg/dl. The mean LDL cholesterol level was 146.6 mg/dl.³⁰

Our study also relates to the context as the newly diagnosed diabetic patients had increased levels of triglycerides (183.15 ± 146.10) and total serum cholesterol (167.86 ± 57.11) and low levels of HDL (38.34 ± 13.64) when compared to healthy individuals. Hence regular monitoring of lipid profile is advised after the diagnosis of diabetes mellitus.

Conclusively, combined dyslipidaemia was seen to be relatively high among newly diagnosed T2DM patients, and in those >40 years. Gender is strongly associated with dyslipidemia associated with T2DM, and men may be at greater risk than women. BMI and family history of diabetes are potential risk factors for dyslipidemia in T2DM. The interesting findings of this study show that multiple dyslipidaemia is common even among newly diagnosed T2DM patients, and this alarming situation necessitates that routine evaluation of lipid profile is ensured on the onset/diagnosis of diabetes.³¹

The main limitation of the present study is the small sample size and short duration of the study that prevents us from concluding the above-stated objectives for a larger population due to which we could not observe the variation in age, gender, smoking status, drinking status, and exercise status in both the groups. Also, our analyses were based on single measurements of Vitamin B12, which may not reflect Vitamin B12 levels over time. Serial changes in serum need to be measured at different stages of T2DM besides newly diagnosed T2DM patients to further clarify the role of Vitamin B12 in the progression of T2DM. Large-scale, long-term randomized- controlled studies are needed to confirm the findings of our study.

CONCLUSION

Our study aimed to evaluate the status of Vitamin B12 in newly diagnosed type 2 diabetic patients and its correlation with the glycaemic status as the primary objective.

As a result, this study shows a significant negative correlation between glycaemic control (FBS, PPBS, and HbA1c) and Vitamin B12 status in newly diagnosed diabetic subjects. Our study implies the fact that Vitamin B12 deficiency might be started in the prediabetic stage and the glycaemic status can start affecting the Vitamin B12 levels in the pre-diabetic stage only or that Vitamin B12 is a potential risk factor in the development of diabetes mellitus.

The diabetic population also had disturbed lipid profiles. The Triglycerides and Total Serum Cholesterol were significantly higher among Diabetic compared to non-Diabetic subjects. And the HDL was significantly higher among non-Diabetic compared to Diabetic subjects. This fact needs more attention and further investigation for better patient care and good healthcare outcomes.

As it has been suggested in various studies that metformin affects the level of Vitamin B12 in prolonged use, and the levels are already degrading due to the diabetic status of the patient, the treatment should be relevant to the fact of regular monitoring and proper supplementation, if needed. Although we found a significant decrease in the level of Vitamin-B12 during the study period, no patients developed Vitamin B-12 deficiency. Careful monitoring of Vitamin B-12 levels is suggested for newly diagnosed and current diabetic patients.

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