Vitamin B12 deficiency and its correlation with lipid profile in Type 2 Diabetes Mellitus patients.

Roshni G. Sadaria, Associate Professor, Department of Biochemistry, Smt. B. K. Shah Medical Institute & Research Centre, SumandeepVidyapeeth University, Piparia, Vadodara
Smita N. Vasava, Assistant Professor, Department of Biochemistry, Smt. B. K. Shah Medical Institute & Research Centre, SumandeepVidyapeeth University, Piparia, Vadodara
Dr. Jasmin Jasani, Professor, Department of Pathology, Smt. B. K. Shah Medical Institute & Research Centre, SumandeepVidyapeeth University, Piparia, Vadodara

ABSTRACT

Background: Type 2 Diabetes mellitus is the leading cause of morbidity and mortality. Vitamin B12 deficiency is associated with multiple neurological and neurocognitive manifestations, including peripheral and autonomic neuropathy in diabetes patients. In the presence of vitamin B12 deficiency, there is an increase in the serum methylmalonic acid (MMA) which leads to defective fatty acid synthesis and dyslipidemia.

Aims: To assess the serum vitamin B12 levels and its correlation with lipid profile in type 2 Diabetes mellitus patients.

Methodology: The present case control study was conducted at Dhiraj General Hospital, Piparia, Vadodara, Gujarat, India in which 100 subjects were enrolled, out of which 50 were cases of type 2 diabetes mellitus patients and 50 were controls. The age group for the study was 35 to 70 years. Blood samples were drawn to measure FBS, HbA1c, vitamin B12 and lipid profile. Interpretation of data was done using Medcalc software.

Results: The mean levels of vitamin B12 was lower in T2DM patients than normal healthy individuals, difference between them was statistically highly significant ($p<0.0001$). The mean levels of serum cholesterol and serum triglyceride were higher in T2DM patients than normal healthy individuals, difference between them was statistically highly significant ($p<0.0001$). The correlations of serum Vitamin B12 levels with other parameters were significant.

Conclusion: From this study, we concluded that there is a decrease in serum Vitamin B12 level. Vitamin B12 is negatively correlated with Cholesterol and Triglyceride and positively correlated with HDL.

Keywords: Type 2 Diabetes Mellitus, Lipid Profile, Vitamin B12.

INTRODUCTION

Diabetes mellitus is a non communicable metabolicdisorder characterized by absolute or relative deficiencies in insulin secretion and/or insulinaction due to either the progressive or marked inability of the β-Langerhans islet cells of the pancreas to produce insulin or due to defects in insulin uptake in the peripheral tissue (insulin resistance). It is associated with chronic hyperglycemia and disturbances of carbohydrate, lipid andprotein metabolism [1,2].

Type 2 Diabetes mellitus is the leading cause of morbidity and mortality. The most common complications of diabetes mellitus are peripheral neuropathy, nephropathy, retinopathy, coronary artery disease, cerebrovascular disease etc[3]. Vitamin B12 deficiency is associated with multiple neurological and neurocognitive manifestations, including peripheral and autonomic neuropathy in diabetes patients [4]. Metformin therapy is now considered a standard first line therapy for type 2 diabetes and is commonly used. Metformin reduces the circulating B12 levels by about 25% [5].Vitamin B12 is a water soluble vitamin. Itexerts its physiological effects through mediating twoprincipal enzymatic pathways i.e. the methylation process of homocysteine to methionine and theconversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA. Vitamin B12 as a co-factor facilitates the methylation of homocysteine to methionine which is later activated into S-adenosyl-methionine that donates its methyl group to methyl acceptors such as myelin, neurotransmitters and membrane phospholipids. Vitamin B12 deficiency hence will result in accumulation of serum homocysteine. Hyperhomocysteinemia hasbeen shown to have potentially toxic effects on neurones and the vascular endothelium. In another essential enzymatic pathway, vitamin B12 as a co-factor mediates the conversion of methylmalonyl coenzyme A (CoA) to succinyl-CoA. In the presence of vitamin B12 deficiency, this conversion pathway is diminished and an increase in the serum methylmalonic acid (MMA) ensues which leads to defective fattyacid synthesis of the neuronal membranes. Therefore, without early detection and treatment, vitamin B12 deficiency can cause irreversible, clinically significant complications and increased morbidity among diabetics [3].

Defects in insulin action and hyperglycemia could lead to changes in plasma lipoproteins in patients with diabetes. In the case of type 2 diabetes, the obesity/insulin-resistant metabolic disarray, the root of this form of diabetes could, itself, lead to lipid abnormalities exclusive of hyperglycemia [6]. Lipid abnormalities in patients with diabetes, often termed "diabetic dyslipidemia", are typically characterized by high total cholesterol (T-Chol), high triglycerides (Tg), low high density lipoprotein cholesterol (HDL-C) and increased levels of small dense LDL particles. Low density lipoprotein cholesterol (LDL-C) levels may be moderately increased or normal [7].

Vitamin B12 may affect serum lipid parameters because it is a cofactor of the enzyme that plays a role in fatty acid catabolism, and also because of its association with obesity and increased risk of myocardial infarction in its deficiency. The purpose of this study is to assess the vitamin B12 levels in type 2 diabetes patients and its correlation with lipid profile on Type 2 Diabetes mellitus patients.

MATERIALS AND METHODS

The present case control study was conducted at Dhiraj General hospital, Piparia, Vadodara, Gujarat, India in which 100 subjects were enrolled, out of which 50 were cases of type 2 diabetes mellituspatients and 50 were controls. Study was conducted from July 2019 to Dec 2019.

Inclusion Criteria

Cases: Patients Diagnosed with Type 2 diabetes mellitus.

Controls: Non Diabetic healthy individuals.

Age group for both cases and controls was 35-70 years.

Exclusion Criteria

Cases:- Those who were not willing to participate.

- DM patients with Liver, Renal or heart disease that could affect the outcome of the study.

- Patients taking vitamin B12 supplementation.

Controls: - Those who were not willing to participate.

- Individuals taking vitamin B12 supplementation.

- Data was collected by personal interview, with every individual. Performa was available for the filling of biodata such as age and gender, clinical examination findings, investigations like Fasting blood glucose, glycated hemoglobin (HbA1c), lipid profile and vitamin B12. Participants were informed of the study purpose.
- We had taken 50 patients diagnosed with Type 2 Diabetes Mellitus as cases and 50 Non Diabetic individuals as controls within the age group of 35-70 years attending the medical outpatient department and inpatient department of Dhiraj General Hospital.
- Written Informed consents were taken in their respected languages and fasting blood was collected in vacutainer tubes.
- Fasting blood sugar (FBS) was estimated by glucose oxidase-peroxidase method on EM-200 fully auto chemistry analyzer.
- HbA1c was done by by HPLC method on HB Vario machine in the Laboratory of Dhiraj General hospital.
- Serum Total cholesterol and Serum Triglyceride were done by Cholesterol oxidase peroxidase & Glycerophosphate oxidase (GPO) end point method respectively. HDL-C was estimated using HDL direct reagent based on modified polyvinyl sulfonic acid (PVS) and PEGME coupled classic precipitation method. Serum VLDL and LDL were calculated by Friedewalds formula. Lipid profile was performed on EM-200 fully auto chemistry analyzer.
- Serum Vit B12 level was measured by Chemiluminescence Immuno assay Method in the Laboratory of Dhiraj General Hospital on Maglumi 800 analyzer.

Statistical Methods

Data was presented as Mean and SD values. Test of significance was assessed by independent t-test. A p-value less than 0.05 ($p < 0.05$) is considered as statistically significant. Medcalc software was used for all statistical analysis. Data was presented in tabulated as well as graphical format.

RESULTS

Results are presented as Mean ± SD. The basic characteristics and mean distribution of biochemical parameters in the cases and controls are depicted in Table 1. There was no significant difference in age between the two groups. Table 1 shows, the mean level of Fasting blood sugar was 212.40 ± 54.92 in cases and 86.82 ± 8.74 in controls. Patients of DM type 2 had significantly higher level of FBS than normal healthy individuals, difference between them was statistically highly significant ($p<0.0001$). The mean level of HbA1c was 9.50 ± 1.69 in cases and 5.17 ± 0.53 in controls. Patients of DM type 2 had significantly

higher level of HbA1c than normal healthy individuals, difference between them was statistically highly significant (p<0.0001). The mean level of serum vitamin B12 was 149.87 ± 37.69 in cases and 547.85 ± 105.17 in controls. Patients of DM type 2 had significantly lower level of serum vitamin B12 than normal healthy individuals, difference between them was statistically highly significant (p<0.0001). The mean level of Cholesterol was 216.30 ± 47.19 in cases and 166.10 ± 27.09 in controls. Patients of DM type 2 had significantly higher level of Cholesterol than normal healthy individuals, difference between them was statistically highly significant (p<0.0001). The mean level of Triglyceride was 188.77 ± 84.11 in cases and 123.57 ± 42.80 in controls. Patients of DM type 2 had significantly higher level of triglyceride than normal healthy individuals, difference between them was statistically highly significant (p<0.0001). Difference of mean HDL values between cases and controls was not significant, though HDL values in cases of DM type 2 were low than the controls.Graph 1, 2, 3, 4, 5 and 6 shows graphical presentation of mean & SD levels of FBS, HbA1c, Vit B12, Cholesterol, Triglyceride and HDL in cases and controls respectively. Table 2 shows correlation between Vit B12 and other biochemical parameters. Vitamin B12 was negatively correlated with FBS, HbA1c and Cholesterol. These correlations were very significant. There was no correlation found between vitamin B12, HDL and Triglyceride. Fig 1,2,3 shows correlation scatter plot of Vitamin B12 with Cholesterol, HbA1c and FBS in cases respectively.

Table 1: Mean distribution of biochemical parameters in DM type 2 cases and controls. Values are expressed as means ±SD.

Parameters	Cases (n=40)	Controls (n = 40)	P value
AGE (years)	56.32 ± 8.37	51.97 ± 11.71	0.06
FBS (mg/dl)	212.40 ± 54.92	86.82 ± 8.74	<0.0001
HbA1C (%)	9.50 ± 1.69	5.17 ± 0.53	<0.0001
Vit B12(pg/ml)	149.87 ± 37.69	547.85 ± 105.17	<0.0001
Cholesterol(mg/dl)	216.30 ± 47.19	166.10 ± 27.09	<0.0001
Triglyceride(mg/dl)	188.77 ± 84.11	123.57 ± 42.80	<0.0001
HDL(mg/dl)	45.82 ± 3.94	47.65 ± 4.92	0.07
LDL(mg/dl)	132.72 ± 48.45	93.73 ± 26.90	<0.0001

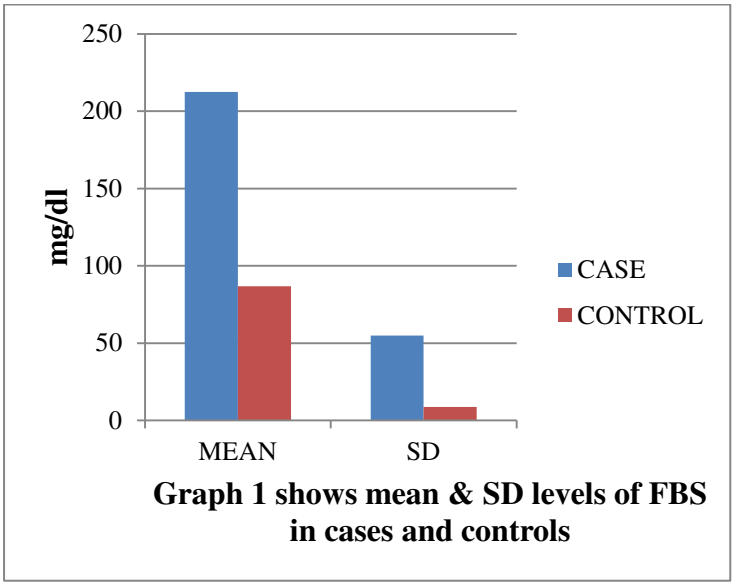
VLDL(mg/dl)	37.75 ± 16.82	24.71 ± 8.56	<0.0001

p < 0.05 - significant, p< 0.001 - very significant p< 0.0001 - highly significant, p ≥ 0.05 - not significant

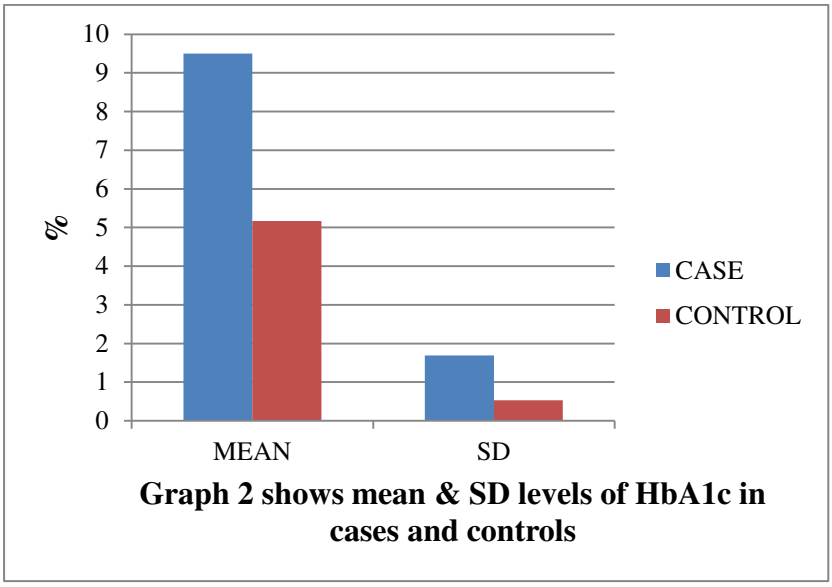
Table 2: Correlation of vitamin B12 with different variables and biochemical parameters among the cases.

Parameters		**Age**	**HbA1c**	**FBS**	**Cholesterol**	**Triglyceride**	**HDL**
VITAMIN B12	**r value**	-0.01	-0.585	-0.492	-0.451	-0.127	0.02
	P value	0.92	<0.001	<0.001	<0.05	0.23	0.90

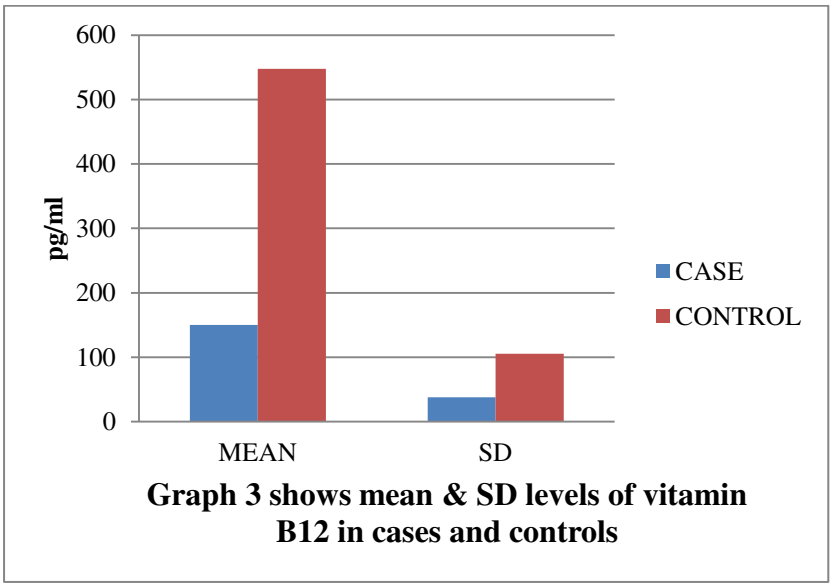
p < 0.05 - significant, p< 0.001 - very significant ,p< 0.0001 - highly significant, p ≥ 0.05 - not significant



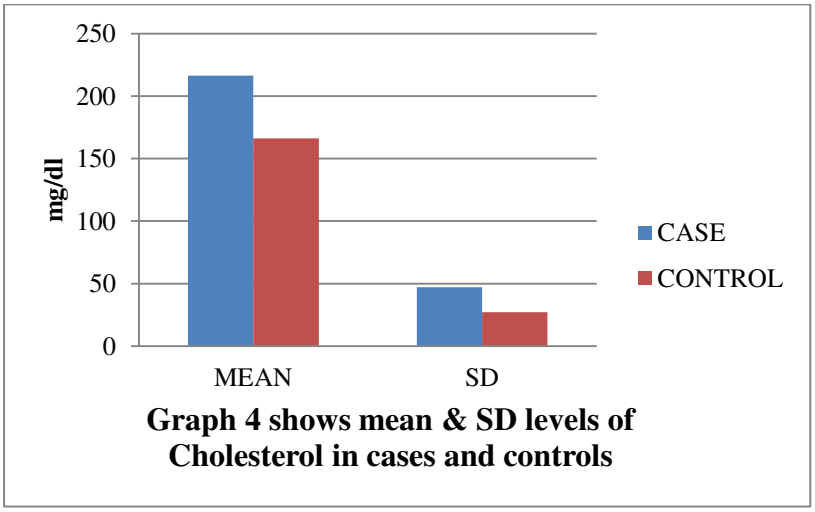
Graph 1 shows mean & SD levels of FBS in cases and controls



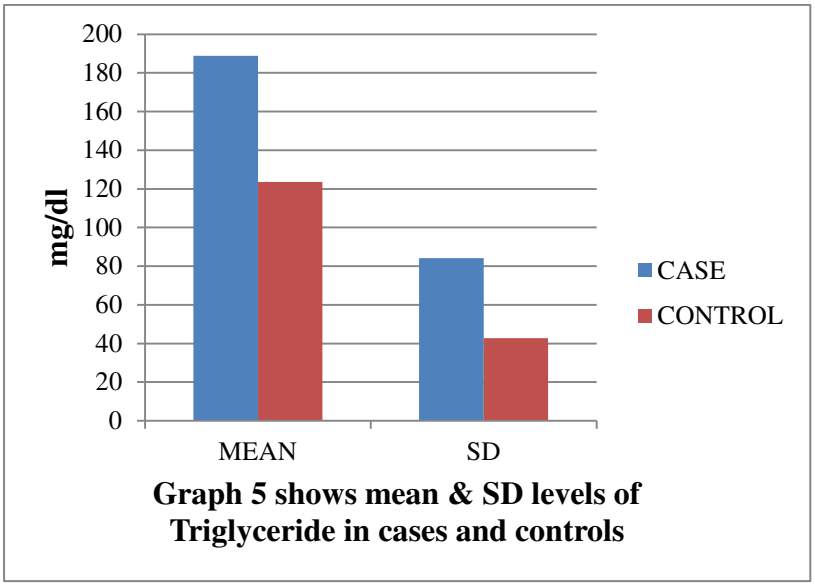
Graph 2 shows mean & SD levels of HbA1c in cases and controls



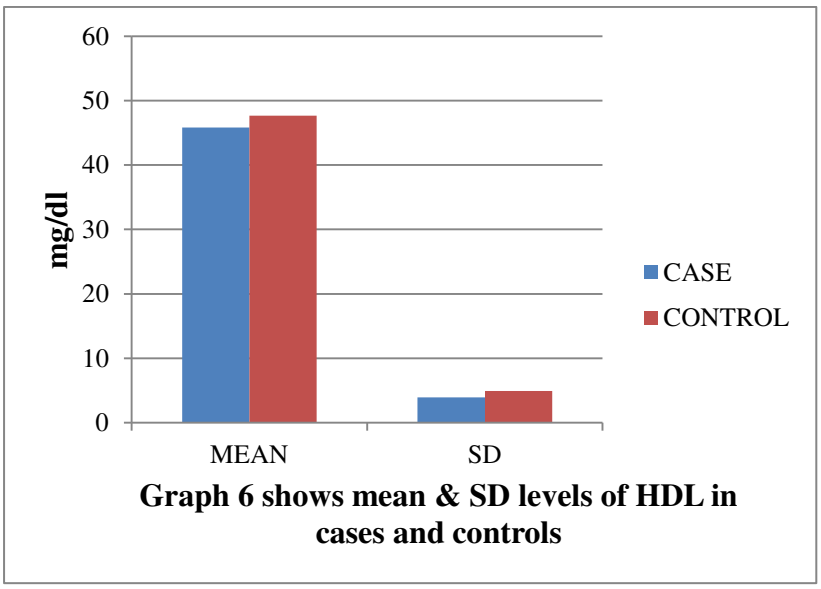
Graph 3 shows mean & SD levels of vitamin B12 in cases and controls



Graph 4 shows mean & SD levels of Cholesterol in cases and controls



Graph 5 shows mean & SD levels of Triglyceride in cases and controls



Graph 6 shows mean & SD levels of HDL in cases and controls

Fig 1: Correlation scatter plot of cholesterol vs. vitamin B12 in cases

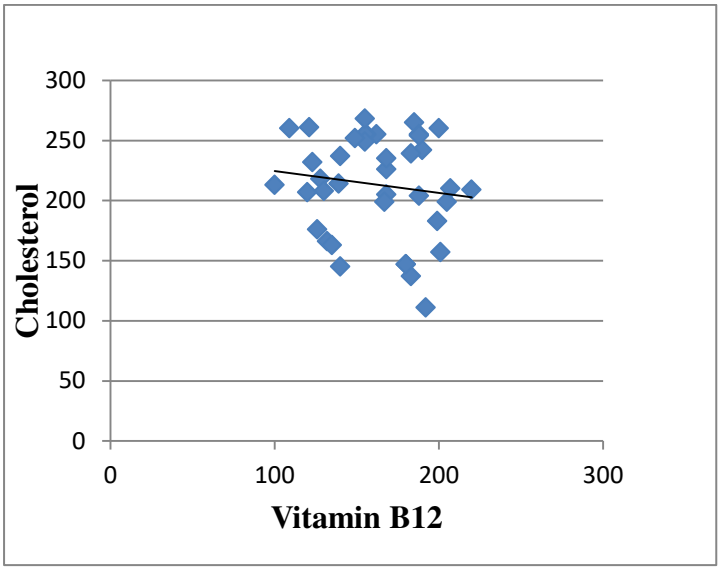


Fig 2: Correlation scatter plot of HbA1c vs. vitamin B12 in cases.

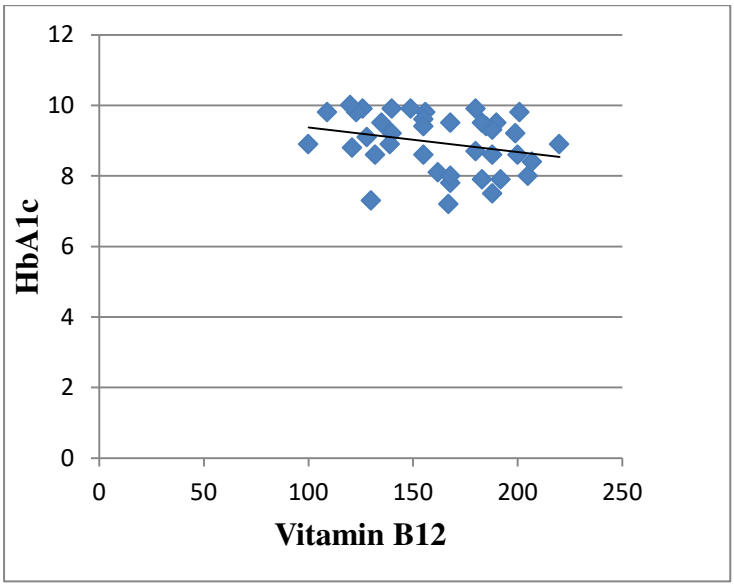
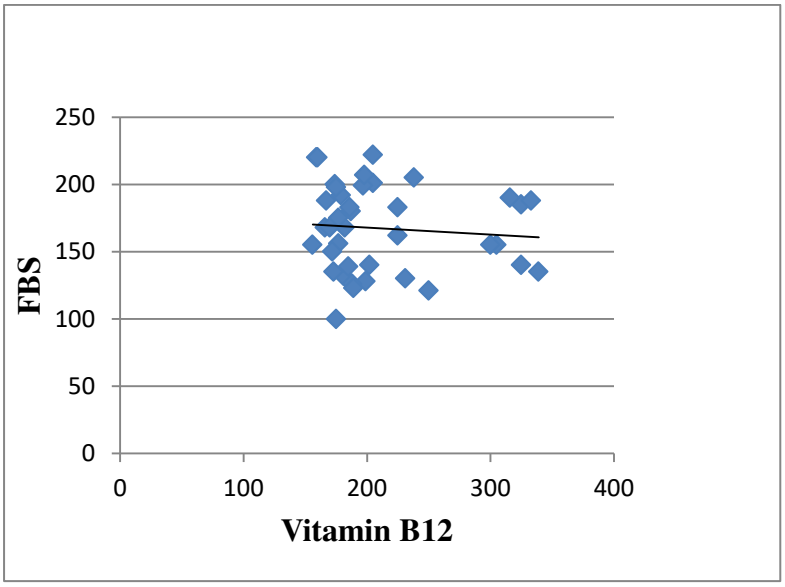


Fig 3: Correlation scatter plot of FBS vs. vitamin B12 in cases.



DISCUSSION:

Type 2 DM, the most common form of DM, results from interaction between genetic, environmental and behavioural risk factors. People living with type 2 DM are more vulnerable to various forms of both short and long-term complications, which often lead to premature death [8]. Peripheral neuropathy is the most common complication of T2DM followed by cardiovascular, renal and ophthalmic complications [9].

In our study we found that patients of DM type 2 had significantly lower level of serum vitamin B12 than normal healthy individuals, difference between them was statistically highly significant. Our findings are coincides with study done by Shamim Ahmed et al[3]. K.S. Akinlade et al has shown that vitamin B12 deficiency and borderline deficiency were recorded in 8.6% and 26.0% of the T2DM patients respectively [10]. One cross-sectional study of 203 type 2 diabetes patients also has reported the prevalence of B12 deficiency is 22% [11]. N. Raizada et al concluded in their study that Metformin use was associated with a lower serumVitamin B12 levels. Metformin drug is used as a first line drug for the treatment of DM type 2 [12]. Other causes of vitamin B12 deficiency might be inadequate intake of nutrition, decreased metabolism and common chronic health problems [13]. Vitamin B12 deficiency will lead to hyperhomocysteinemia. Homocysteine can be remethylated to methionine or transsulphurated to cystathionine. Former requires 5-methyltetrahydrofolate as methyl donor and vitamin B12 as a co-factor. Hyperhomocysteinemia (HHcy) is a risk factor strongly linked to cardiovascular complications in T2DM [9].

High T-Chol, high Tg and low HDL-C in type 2 DM patients were found in study done by Bhowmik B [7]. Our study findings are coincides with them.Shyamala K. Venkatesh et al also showed that the mean value of TC, VLDL and LDL were higher in overall T2DM patients than the normal range and HDL was lower in T2DM patients [14]. A study done by Gordon L et al showed that lipid and lipoprotein profiles of type 2 diabetic patients were not statistically different from those of non-diabetic subjects [15].

Sezgin Y et al concluded that there is a relationship between vitamin B12 and serum lipid parameters, and that serum triglyceride levels were especially affected by vitamin B12 treatment. [16]. Vitamin B12 functions as a coenzyme in the conversion of methylmalonyl CoA (MM-CoA) to succinyl-CoA. This reaction is blocked if there is vitamin B12 deficiency, resulting in accumulation of MM-CoA which inhibits the rate-limiting enzyme of fatty acid oxidation (CPT1 – carnitine palmitoyl transferase), thus causing lipogenesis. This may be the likely mechanism for the link between B12 deficiency and adverse lipid parameters [5,16]. In our study we found that Vitamin B12 was negatively correlated with FBS, HbA1c and Cholesterol. These correlations were very significant. There was no correlation found between vitamin B12, HDL and Triglyceride.Namita Mahalle et al also showed that serum vitamin B12 was inversely associated with VLDL and positively with HDL [17]. In contrast to all this studies, Gunn Helen Moen et al concluded that there was no evidence that serum concentrations of vitamin B12 were casually related to glycated hemoglobin, triglycerides, coronary artery disease, or HDL,LDL or total cholesterol [18].

CONCLUSION:

In our study we found Vitamin B12 is negatively associated with Cholesterol and Triglyceride, and positively associated with HDL. Vitamin B12 deficiency may lead to hyperhomocysteinemia which itself is a cardiovascular risk factor. Vitamin B12 deficiency may lead to dyslipidemia also. Routine screening of vitamin B12 in patients with Type 2 Diabetes Mellitus will significantly reduce the morbidity and mortality in these patients.

REFERENCES

1. Ozder. Lipid profile abnormalities seen in T2DM patients in primary healthcare in Turkey: A cross-sectional study. Lipids in Health and Disease. 2014;13:183.

2. Antwi-Baffour, S., Kyeremeh, R., Boateng, S.O. et al. Haematological parameters and lipid profile abnormalities among patients with Type-2 diabetes mellitus in Ghana. Lipids Health Dis **17,** 283 (2018). https://doi.org/10.1186/s12944-018-0926-y.
3. Shamim Ahmed, SyedaMohsina Rohman. Study of serum Vitamin B12 and its correlation with Lipid profilein Type 2 Diabetes Mellitus. IJBAMR. Sep 2016;5(4):92-103.
4. M Alvarez, Oswaldo Rincón Sierra**,** et al.Vitamin B12 deficiency and diabetic neuropathy in patients taking metformin: a cross-sectional study.Endocrine Connections (2019) 8, 1324–1329.
5. Adaikalakoteswari et al.: Vitamin B12 deficiency is associated with adverse lipid profile in Europeans and Indians with type 2 diabetes. Cardiovascular Diabetology 2014 13:129.
6. Ira J. Goldberg, Diabetic Dyslipidemia: Causes and Consequences, The Journal of Clinical Endocrinology & Metabolism, Volume 86, Issue 3, 1 March 2001, Pages 965–971, https://doi.org/10.1210/jcem.86.3.7304.
7. Bhowmik B, Siddiquee T, Mujumder A, et al. Serum Lipid Profile and Its Association with Diabetes and Prediabetes in a Rural Bangladeshi Population. Int J Environ Res Public Health. 2018;15(9):1944.
8. Abdulfatai B. Olokoba, Olusegun A. Obateru, et al. Type 2 diabetes mellitus: A review of current trends. Oman Medical Journal. 2012;27(4):269-273.
9. Twinkal R. Upadhyay, Nitin Kothari, et al. Association Between Serum B12 and Serum Homocysteine Levels in Diabetic Patients on Metformin. Journal of Clinical and Diagnostic Research. 2016 Apr,10(4):1-4.
10. Akinlade KS, Agbebaku SO, Rahamon SK, Balogun WO. Vitamin b12 levels in patients with type 2 diabetes mellitus on Metformin. Ann Ibd. Pg. Med. 2015;13(2):79-83.
11. Pflipsen MC, Oh RC, Saguil A, Seehusen DA, Seaquist D, Topolski R: The prevalence of vitamin B12 deficiency in patients with type 2 diabetes: a cross-sectional study. J Am Board Fam Med 2009, 22(5):528–534.
12. Raizada N, Jyotsna VP, Sreenivas V, Tandon N. Serum vitamin B12 levels in type 2 diabetes patients on metformin compared to those never on metformin: A cross-sectional study. Indian J Endocr Metab 2017;21:424-8.
13. Appold K. Dangers of Vitamin B12 Deficiency. Journal of Geriatric Medicine. ; 5(1): p. 30.
14. Shyamala K. Venkatesh, Sudheer K. M. V., et al. Lipid profile analysis of type 2 diabetic patients in Bengaluru population, India. International journal of research in medical sciences.2018;6(6).

15. Gordon L, Ragoobirsingh D, Morrison EY, Choo-Kang E, McGrowder D, Martorell E. Lipid profile of type 2 diabetic and hypertensive patients in the jamaican population. J Lab Physicians. 2010;2(1):25–30. doi:10.4103/0974-2727.66709.
16. Sezgin Y, Becel S. Evaluation of Lipid Parameters in Patients Receiving Vitamin B12 Therapy. İstanbul Med J 2019; 20(3): 214-7.
17. Namita mahalle, Mohan Kulkarni, et al. Vitamin B12 deficiency and hyperhomocysteinemia as correlates of cardiovascular risk factors in Indian subjects with coronary artery disease. Journal of Cardiology. April 2013;61(4):289-94.
18. Gunn-Helen Moen, Elisabeth Qvigstad, Kåre I Birkeland, David M Evans, Christine Sommer, Are serum concentrations of vitamin B-12 causally related to cardiometabolic risk factors and disease? A Mendelian randomization study, The American Journal of Clinical Nutrition, Volume 108, Issue 2, August 2018, Pages 398–404, https://doi.org/10.1093/ajcn/nqy101