Original article:

Study of serum Vitamin B12 and its correlation with Lipid profilein Type 2 Diabetes Mellitus

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Abstract:

Introduction: Diabetes Mellitus is a leading cause of death worldwide. The most common complications of diabetes mellitus are peripheral neuropathy, nephropathy, retinopathy, coronary artery disease, cerebrovascular disease etc. Vitamin B12, a water soluble exerts its physiological effects through the methylation of myelin sheath and vitamin B12 deficiency hence will result in disruption of the methylation process and accumulation of intracellular and serum homocysteine which is potentially toxic to the neurones and the vascular endothelium.

Present study was undertaken to assess and compare level of serum Vitamin B12 in patients with type 2 diabetes mellitus with the controls and to find out its correlation with lipid profile.

Materials and methods: The study consisted of 80 patients with type 2 DM, admitted in Gauhati Medical College and Hospital and 40 healthy individuals as controls. Serum Vitamin B12, Fasting plasma glucose, HbA1c and Serum lipid profile were estimated in all the groups.

Results: All the parameters, FBS, HbA1, cholesterol, triglyceridelevels were found to be increased in the patients of Type 2 DM as compared to controls except Vitamin B12 and HDL. The correlation of mean serum Vitamin B12levels with other parameters were significant .

Conclusion: From the present study, it is concluded that there is an decrease in serum Vitamin B12 level with increase in HbA1c ,Cholesterol and triglyceride levels.

Keywords: Type 2 Diabetes mellitus, Fasting Plasma Glucose, Glycated hemoglobin.

Introduction

Diabetes mellitus is a disorder of carbohydrate metabolism in which there is hyperglycemia due to insulin resistance or defective insulin action or both. Type 2 Diabetes mellitus is the leading cause of morbidity and mortality. Diabetes is rapidly gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. In 2000, India topped the world with the highest number of people with diabetes mellitus (31.7 million) followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively (WHO). It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India (WHO)[1]

The most common complications of diabetes mellitus are peripheral neuropathy, nephropathy, retinopathy, coronary artery disease, cerebrovascular disease etc. Among these, the peripheral neuropathy is the commonest and earliest complication in diabetics.Vitamin B12 is a water soluble vitamin. It exerts its physiological effects through mediating two principal enzymatic pathways i.e. the methylation

process of homocysteine to methionine and the conversion 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. Metabolically significant vitamin B12 deficiency hence will result in disruption of the methylation process and accumulation of intracellular and serum homocysteine. Hyperhomocysteinemia has been shown to have potentially toxic effects on neurones and the vascular endothelium. This reaction is also essential in the conversion of dietary folate (methyl-tetrahydrofolate) to its active metabolic form, tetrahydrofolate. 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. This is followed by defective fatty acid synthesis of the neuronal membranes[2]. Vitamin B12 is also essential in the synthesis of monoamines or neurotransmitters like serotonin and dopamine[3]. This synthesis is impaired with vitamin B12 deficiency. Therefore, without early detection and treatment, vitamin B12 deficiency can cause irreversible, clinically significant complications and increased morbidity among diabetics.

Materials and methods

The study was conducted at the department of Biochemistry, Gauhati Medical College & Hospital. It was a cross sectional study involving 50 patients with diagnosed Type 2DM in the age group of 40-80 years of either sex, attending Out Patient Department of Medicine and Endocrinology, GMCH as case and 50 age and sexmatched randomly selectednormal healthy as controls. The study was conducted as per the guidelines of institutional Ethics Committee and informed consent were obtained from all the participants.

Inclusion criteria- 50 cases of T2DM diagnosed as per standard criteria given by World Health Organization.

Exclusion Criteria- Patients with the following diseases were excluded –

1) Patients with type 1 DM

2) Patients with thyroid disorders,Hemolytic Anemia, Hemoglobin variants, Pregnancy,Hepatic diseases and Infectious diseases

Sample collection- 8 hours fasting sample was collected from the Cases and controls. Vitamin B12 and lipid profile were estimated in serum and FBS and HBA1c were estimated in plasma.Sample analysis- Estimations of Fasting plasma glucose, Glycated hemoglobin, cholesterol, triglyceride and HDL were done using MERCK microlab 300 Semiautoanalyser. VLDL and LDL calculated using Friedwald's formula.Serum Vitamin B12 was estimated by ELISA Microplate Reader (Biorad Model 680). The results obtained were statistically analyzed and compared between the two groups of the study. Baseline characteristics of the study participants are expressed in Mean ± SD. Paired student-t test were used to analyze differences in baseline characteristics between the studied groups. Correlations were observed by using Pearson's correlation coefficient. The results were considered significant when the probability (p value) was less than 0.05 of the observed values of "t" at a particular degree of freedom. Statistical analysis was done using GraphPadInStat version 3.00. All the statistical graphs were prepared using Microsoft Excel 2007.

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Results

The age distributions in the two groups were 52.15±8.66 and 54.14±7.14 respectively in controls and cases which were statistically matched (Table 1). Among the cases 40% were female and 60% were male and in the control group 30% were female and 70% were male (Table 1).

The means of vitamin B12 in controls and cases were 597.47 and 404.36 respectively and it was significantly low in cases (p<0.0001) (Table 2).

It was found that, among the cases, prevalence of vitamin B12 deficiency was 10%, borderline deficiency was 17.5% (Table 3).

Vitamin B12 deficiency was significantly associated with increasing age (p<0.05), increased duration of diabetes (p<0.05)(Table 4).

The mean of FPG in Controls and cases were 90.68 and 204.15 respectively and the difference was extremely significant (p<0.0001) (Table 2).

The mean of HbA1c was 5.1 in controls and 8.49 in cases and it was extremely significantly high in cases (p<0.0001) (Table 2).

Lipid profile derangement was an obvious feature in the present study among the cases with type2 DM. Total chol-esterol and triglyceridewere significantly elevated (p<0.0001) but HDL-Cholesterol was reduced among diabetics when compared to non-diabetic controls (Table 2).

The Pearson's correlation of Vitamin B12 with the other variables (age, disease duration) and biochemical parameters (FPG, HBA1c, Cholesterol,Triglyceride) showed significant negative correlation but a significant positive correlation with HDL-cholesterol (r = 0.310) (Table 5).

	Controls (mean±SD)	Cases(mean±SD)	p value
Mean age	52.15±8.66	54.14±7.14	0.61
Sex(Male/Female)	24/16	28/12	0.810

Table 1 : Age & sex distribution of controls and cases

Parameters	Control (mean±SD)	Case (mean±SD)	p value
FPG(mg/dl)	90.68±10.19	204.15±85.50	<0.0001
HbA1C% (NGSP)	5.10±0.29	8.49±2.22	<0.0001
CHOLESTEROL(mg/dl)	138.22±12.92	207.45±45.78	<0.0001
TRIGLYCERIDES(mg/dl)	129.90±21.51	146.68±31.28	<0.05
HDL (mg/dl)	48.17±8.87	36.37±5.86	<0.0001
LDL (mg/dl)	108.01±21.13	135.62±46.07	0.07
VLDL (mg/dl)	27.14±4.34	33.35±6.52	0.053
VITAMIN B12 (pg/ml)	597.47±203.24	404.36±180.28	<0.0001

*significant(<0.05), **very significant(<0.001), *** extremely significant(<0.0001), NS Not significant

Table 2: Showing comparisons of biochemical parameters of the control and case groups. Paired student t-test was used for comparison of means between the two groups

	Controls (Prevalence %)	Cases (Prevalence %)	p value
Vitamin B12 <200 pg/ml (Deficiency)	0 (0%)	4 (10%)	0.113
Vitamin B12 200-300 pg/ml (Borderline)	3 (7.5%)	7 (17.5%)	0.312
Vitamin B12 >300 pg/ml(Normal)	37 (92.5%)	29 (72.5%)	0.036

Table 3: prevalence of vitamin B12 deficiency in study groups

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Continuous variables		Vitamin B12			p Value
		Deficiency	Borderline Deficiency	No deficiency	
Age(Mean±SD)		62.25±6.29	57.57±3.60	52.73±7.20	0.018
Sex	Male	3	7	18	0.141
	Female	1	0	11	
Disease duration(mean±SD)		11.75±2.5	10.43±2.94	6.24±3.74	0.0024

Table 4: Association vitamin B12 levels and other continuous variables among cases

Parameters	Vitamin b12	
	r value	p value
Age	-0.407	<0.05
Duration of diabetes	-0.738	<0.0001
FPG	-0.513	<0.001
HbA1c	-0.561	<0.001
Cholesterol	-0.399	<0.05
Triglyceride	-0.260	0.105
HDL	0.310	<0.05

Table 5: correlation of vitamin B12 with different variables and biochemical parametrs among the cases

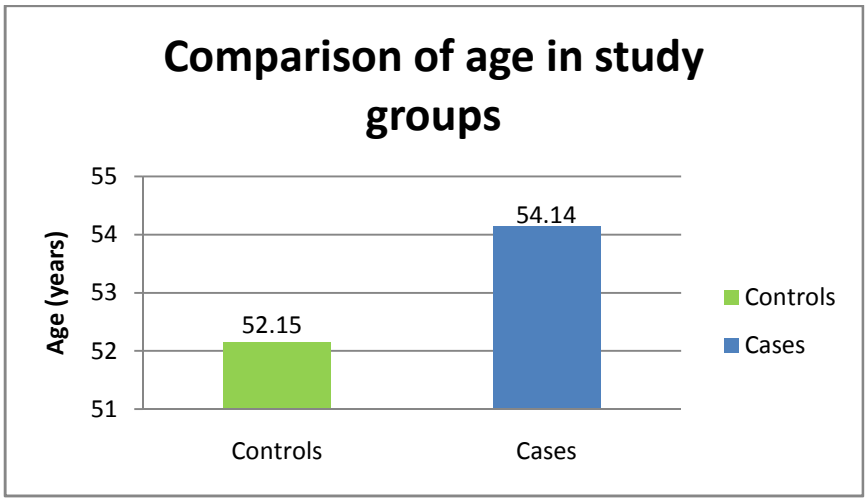

Figure 1: showing comparison of mean age in controls and cases

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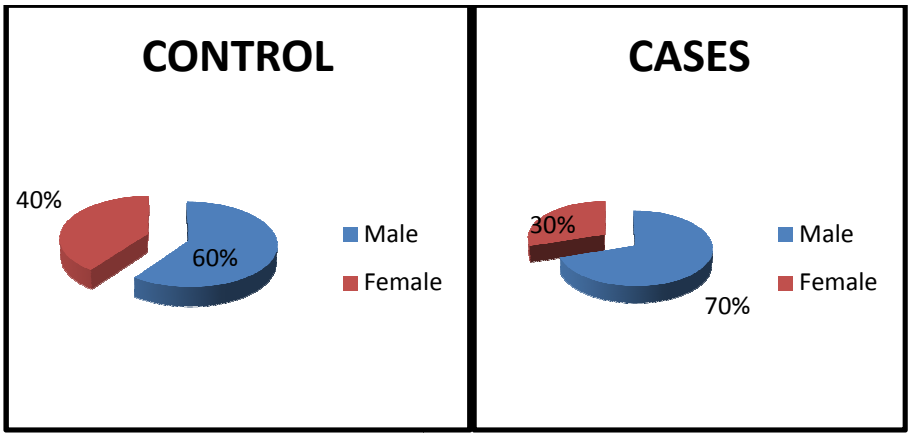

Figure 2: sex distribution in the controls and cases

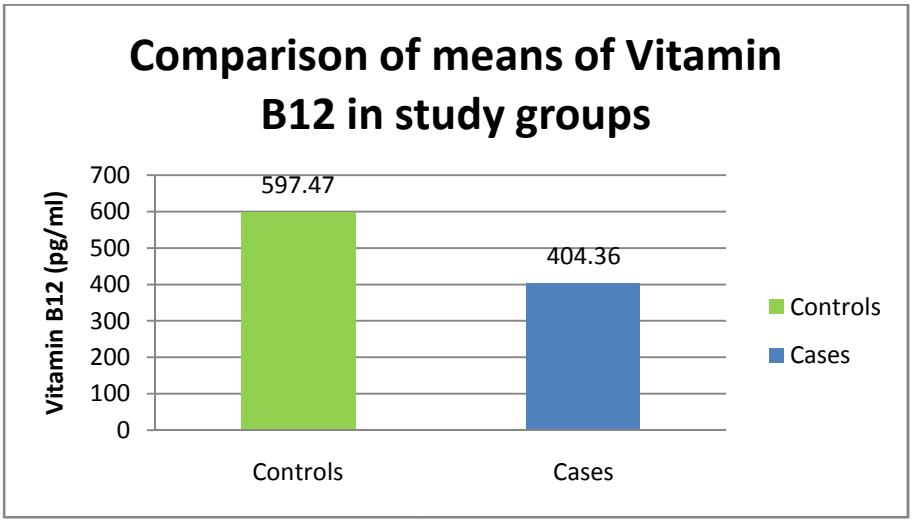

Figure 3: comparison of mean vitamin B12 in controls and cases

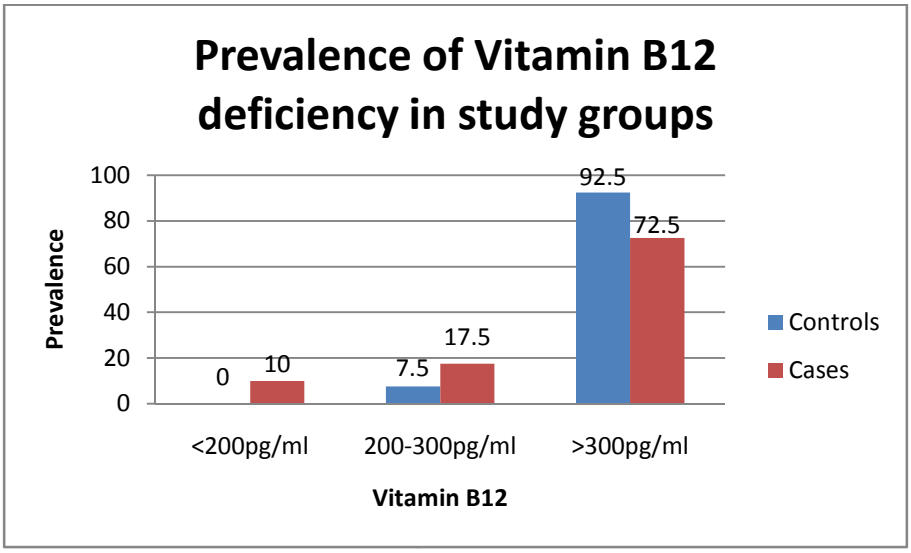

Figure 4: prevalence of vitamin B12 deficiency in controls and cases

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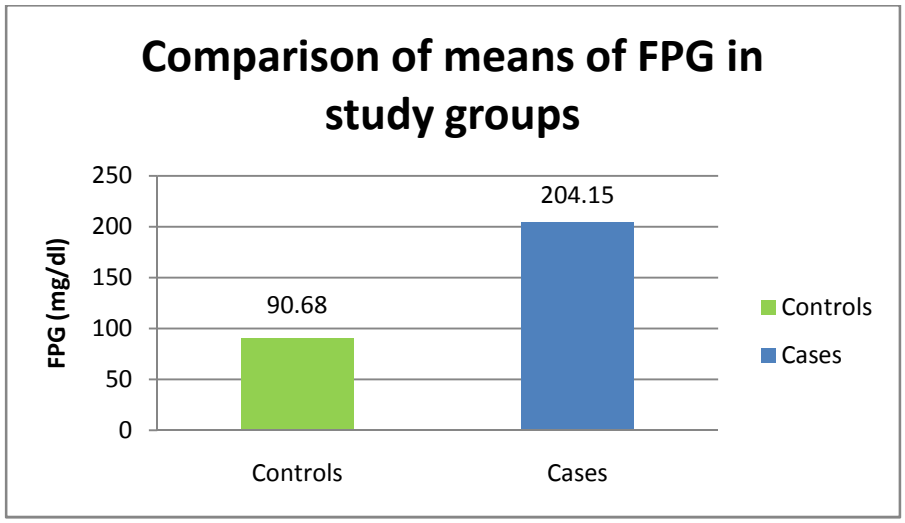

Figure 5: Showing mean FPG in the controls and cases.

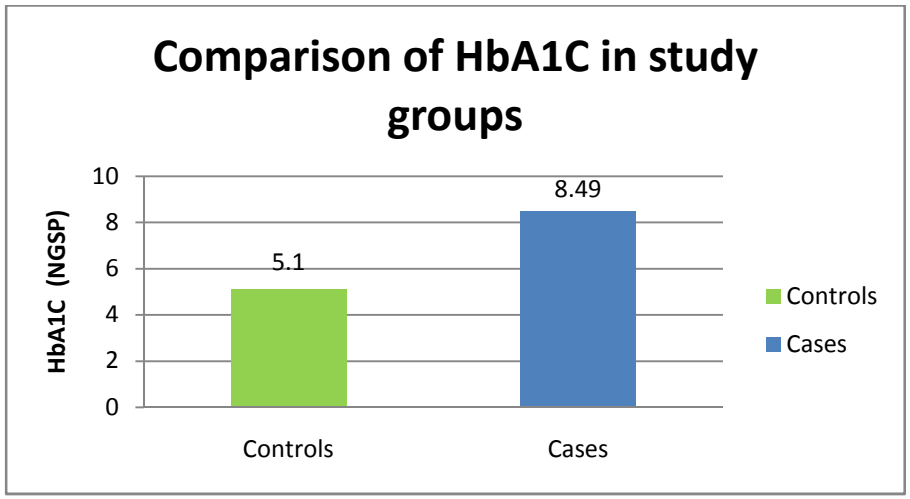

Figure 6: comparison of mean HbA1c in controls and cases

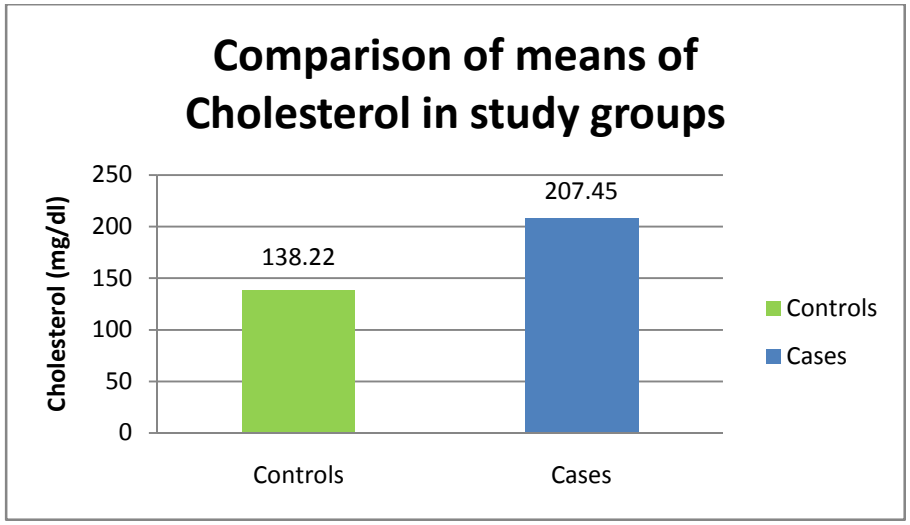

Figure 7: comparison of mean cholesterol in controls and cases

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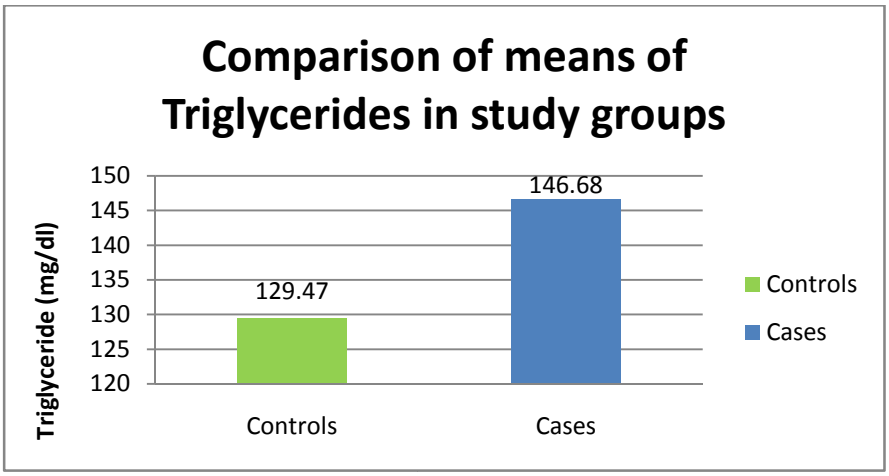

Figure 8: comparison of mean triglyceride in controls and cases

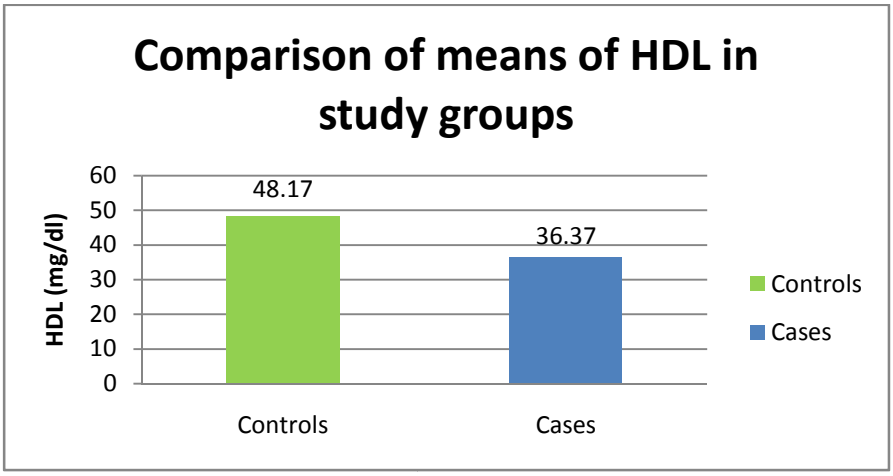

Figure 9: comparison of mean HDL in controls and cases

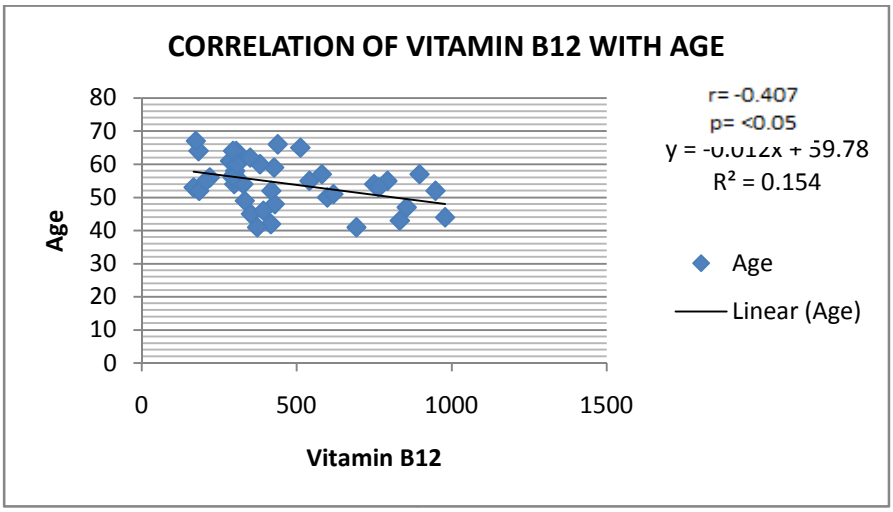

Figure 10:correlation of vitamin B12 with Age in Diabetic patients

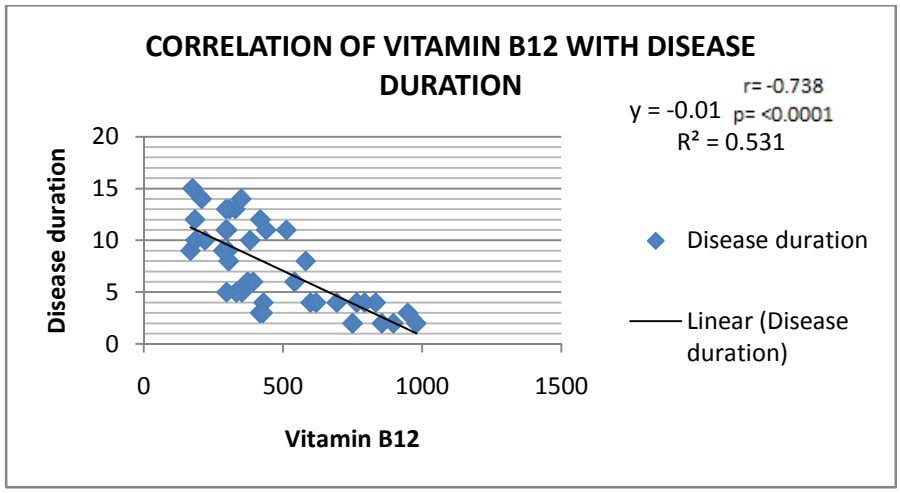

Figure 11: correlation of vitamin B12 with disease duration in Diabetic patients

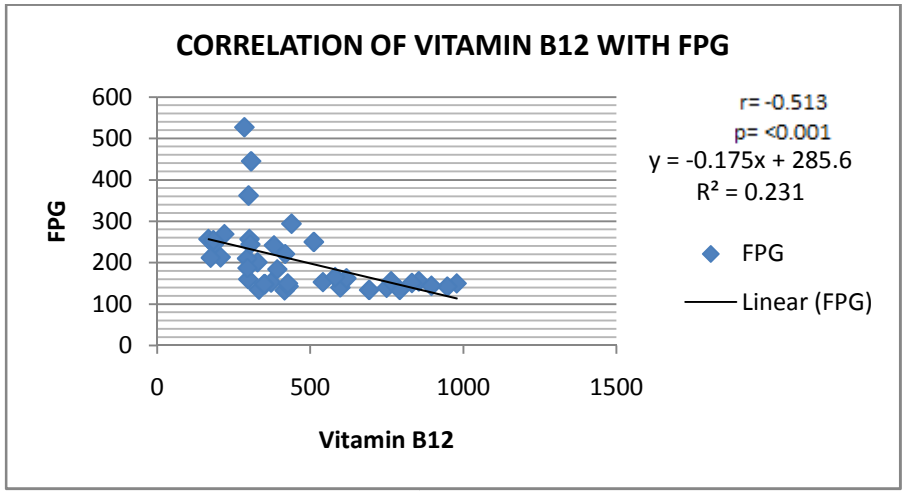

Figure 12: correlation of vitamin B12 with FPG in Diabetic patients

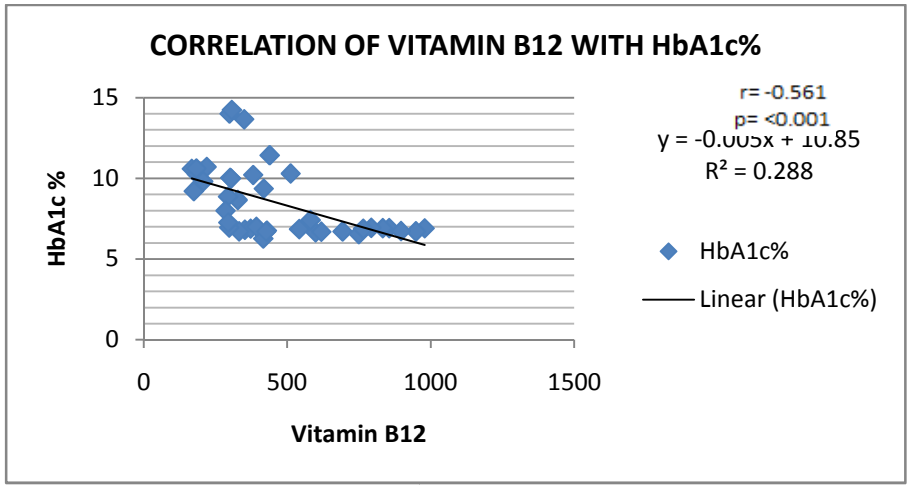

Figure 13:correlation of vitamin B12 with HbA1c% in Diabetic patients

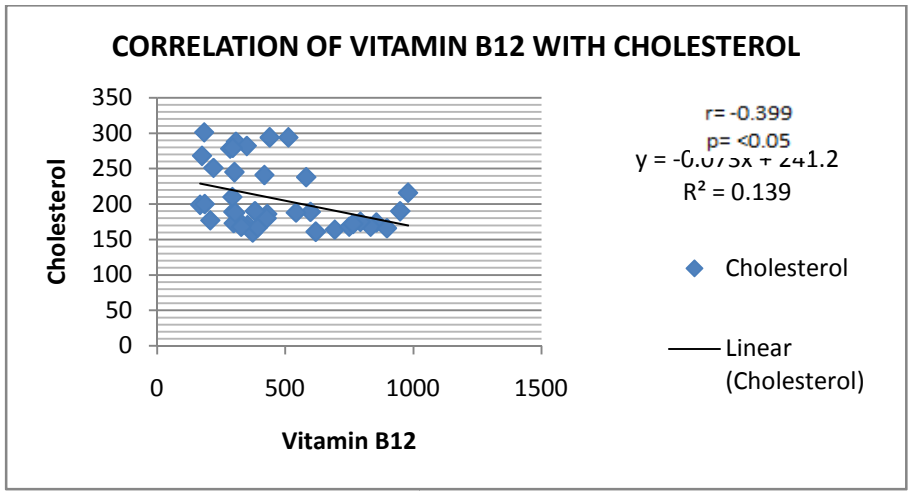

Figure 14: correlation of vitamin B12 with Total cholesterol in Diabetic patients

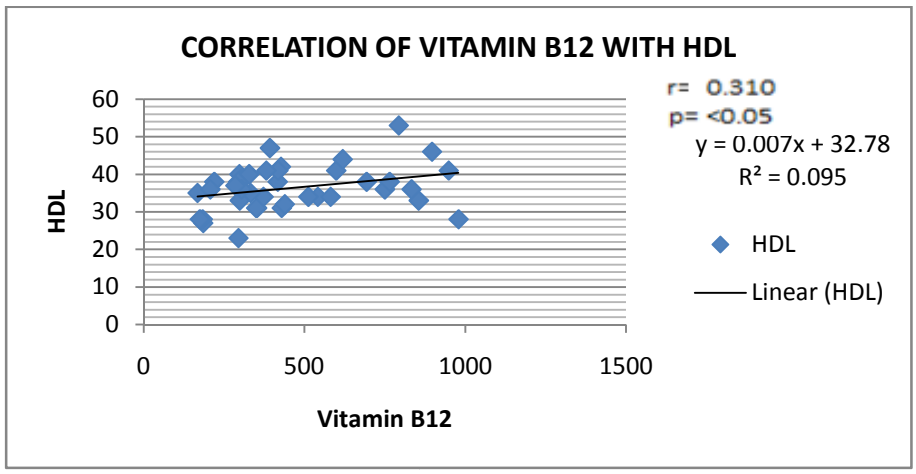

Figure 15: correlation of vitamin B12 with HDL in Diabetic patients

Discussion

Diabetes mellitus, a common endocrine disorder and the complications arising from the disease are the third leading cause of death worldwide. It is a condition where the cells of the body cannot metabolize sugar effectively due to a total or relative lack of insulin. The body then breaks down its own fats, proteins and glycogen resulting in high levels of blood glucose (hyperglycemia), because without insulin cellular uptake and utilization of glucose is limited[4]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.Diabetes Mellitus is a major contributor to morbidity and mortality in society due to lack of proper management and treatment of accompanying complications.

In the present study, the age distribution of the patients was between 40 and 70 years with mean age of cases was 54.14 ±7.14 years. The finding of the present study was in corroboration with the studies by *Ekpenyong C.E.* et al[5] and *Wilson P.W. F.* et al[6] where theyfound the mean age of diabetic patients as

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49.8±0.34 years and 54.0±9.8 years respectively. *Choi B.C.*et[a7l] and *Becker J.*et al[8]also found greater age in diabetic patients compared to controls. In our study, we found most of the subjects were in the age group of 51 to 60 yrs with a relative frequency of 0.50 in case group. *Becker J.*et al[8] also found most of the diabetic patients at the age group of 41-60 years. This reflects that DM is mainly a disease of the aged.Lipid profile derangement was an obvious feature in the present study among the case group. Total cholesterol and triglyceride were significantly elevated (p<0.0001) and HDL-Cholesterol was significantly reduced (p<0.0001) when compared to controls and among diabetics when compared to non-diabetic controls. These findings corroborated with the study conducted by *Samatha P.*et al[9] and *Uttra K.M.* et al[10].Association between the elevated total cholesterol and insulin resistance was also shown statistically significant in the study conducted by *Menik*et al[11]In a study by *RaniH.S.*et al[12], an attempt has been made to evaluate the risk factors for coronary heart disease in DM patients and was observed that FBS, PPBS, total cholesterol, triglyceride VLDL and LDL were high and the levels of HDL were low compared to controls.

In our study, the mean value of serum vitamin B12 in the case group was 404.36±180.28 and it was significantly lower than the control group(p<0.0001). This finding was similar with the findings by *Reinstatler L.* et al[13] and *Pflipsen M.C.* et al[14]. In our study, among the diabetic patients, 10% were having vitamin B12 deficiency (<200pg/ml) and 17% were with borderline deficiency (200-300pg/ml). The deficiency was significantly more in the patients with long duration of diabetes with aaverage duration of 11.75 years. *Pflipsen M.C.* et al[14] showed 22% of diabetic patient as having vitamin B12 deficiency, considering vitamin B12 level<100pg/ml as deficiency, 100-350pg/ml as borderline and >350pg/ml as normal. In their study, they too found the deficiency more in patients with longer duration. It may be due to higher insulin resistance and long term use of metformin[15].The negative correlation of vitamin B12 with age may be explained by inadequate intake of nutrition, decreased metabolism and common chronic health problems effecting the absorption of vitamin B12[16].

The correlation of vitamin B12 with FPG and HbA1c% may be explained by the dominant role of Vitamin B12 in the utilization of carbohydrates.The hypothesis that vitamin B12 is important for carbohydrate or fat metabolism is supported by various experimental facts[17,18,19]. It is found that the erythrocytes of vitamin B12-deficient individuals contain less glutathione or enzyme activities essential for the degradation of glucose to ribose[20,21].Vitamin B12 deficiency causes an elevation of the coenzyme A content in the liver[22] marked decrease in the reduced form of DPN with the concomitant increase in the oxidized form in the liver[23]. Since glutathione, CoA and DPN systems are involved in many metabolic pathways, vitamin B12 deficiency may hamper in the maintenance of balanced enzyme systems essential for the utilization of carbohydrate and the transformation of fat. The negative correlation of total cholesterol and positive correlation of HDL-cholesterol with vitamin B12 in the present study was corroborated with the findings with *Mahalle N.* et al[24] and *Diakoumopoulou E.* et al[25] .Vitamin B12 deficiency leads to accumulation of homocysteine resulting in high levels of S-adenosyl-*L*-homocysteine which is an inhibitor of S-adenosyl-L-methionine dependent methyltransferases. This will cause hypomethylation of various enzymes and

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accumulation of lipids in liver and muscles along with increased level of triglyceride leading to obesity[26,27].

Conclusion

Diabetes mellitus is a group of metabolic disorders of carbohydrate metabolism in which glucose is underused, producing hyperglycemia. There is either insulin resistance or defective insulin action or both. Type 2 Diabetes mellitus is the leading cause of morbidity and mortality. Prevention of Diabetes and its associated burden, primarily Neuropathy and cardiovascular morbidity and mortality has become a major health issue worldwide.Vitamin B12 is a water soluble vitamin that exerts its physiological effects through mediating two principal enzymatic pathways i.e. as cofactor it 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 and also converts methylmalonyl coenzyme A (CoA) to succinyl-CoA. Hence vitamin B12 deficiency will lead to accumulation of intracellular and serum homocysteine. Hyperhomocysteinemia has been shown to have potentially toxic effects on neurones and the vascular endothelium resulting in neuropathy and cardiovascular diseases. Vitamin B12 is an essential micronutrient required in the synthesis of monoamines or neurotransmitters like serotonin and dopamine, DNA synthesis, cellular repair and normal hemopoesis.

From the present study,

- It has been found that the patients with Type 2 diabetes Mellitus have low levels of Serum Vitamin B12, which may be the cause of their various complications during the disease course.
- An attempt was made to find the correlation between Serum Vitamin B12 and FPG, HbA1c% and Lipid profile parameters. There were significant negative correlation with all these parameters except HDL in the case group. We propose that this effect most probably results from the vitamin B12 deficiency that effected the enzyme systems essential for the metabolism of carbohydrate and transformation of fats.
- The extension of the study may also provide an insight into a better understanding on the role of vitamin B12 in the neuronal and cardiovascular protection and to assess the extent of complications in diabetic patients. Routine screening of vitamin B12 in patients with Type 2 Diabetes will significantly reduce the morbidity and mortality in these patients.

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