



Estimation of Serum Homocysteine and Vitamin B₁₂ in Type 2 Diabetes Mellitus Patients

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Authors' contributions

This work was carried out in collaboration among all authors. Author RGS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SNV managed the analyses of the study. Authors JDB, VJB, MB, PC and MC managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Diabetes mellitus is a common metabolic disorder characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism. Homocysteine (Hcy) is a risk factor strongly linked to cardiovascular complications in Type 2 Diabetes mellitus (T2DM). Hcy requires 5-methyltetrahydrofolate as methyl donor and vitamin B12 as a co-factor. So, Vitamin B12 deficiency will lead to hyperhomocysteinemia. Biochemical and clinical vitamin B12 deficiency has been demonstrated to be highly prevalent among patients with type 1 and type 2 diabetes mellitus.

Aims: To assess the serum homocysteine and vitamin B12 levels in type 2 Diabetes mellitus patients.

Methodology: The present case control study was conducted at Dhiraj hospital, Piparia, Vadodara, Gujarat, India in which 80 subjects were enrolled, out of which 40 were cases of type 2 diabetes

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mellitus patients and 40 were controls. The age group for the study was 35 to 70 years. Blood samples were drawn to measure RBS, HbA1c, serum homocysteine and vitamin B12. Interpretation of data was done using Medcalc software.

Results: The mean levels of serum homocysteine was higher in T2DM patients than normal healthy individuals, difference between them was statistically highly significant ($p < 0.0001$). The mean levels of vitamin B12 was lower in T2DM patients than normal healthy individuals, difference between them was statistically highly significant ($p < 0.0001$). There is no correlation found between Homocysteine, vitamin B12 & HbA1c.

Conclusion: A significant increase in serum Hcy level was observed in T2DM patients in our study. Raised serum Homocysteine is considered as an early marker of B12 deficiency. Hyperhomocysteinemia will lead to cardiovascular complications. Therefore, the hyperhomocysteinemia could serve as another important marker of poor diabetic control and developing complications.

Keywords: Homocysteine; type 2 diabetes mellitus; vitamin B12.

1. INTRODUCTION

Diabetes mellitus is a common metabolic disorder characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid and protein metabolism [1]. 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 their premature death [2]. Type 2 Diabetes Mellitus (T2DM) accounts for 90% of global diabetic population and 9% of annual global mortality with health and socioeconomic problems [3].

Homocysteine has been under a lot of speculation since its discovery in 1932. Its chemical properties showed a similarity to cysteine, hence the name homocysteine. Homocysteine, a sulfhydryl-containing amino acid, is an intermediate product in the normal biosynthesis of the amino acids methionine and cysteine [4]. Homocysteine (Hcy) is a risk factor strongly linked to cardiovascular complications in T2DM. Hcy can be remethylated to methionine or transsulphurated to cystathionine. Former requires 5-methyltetrahydrofolate as methyl donor and vitamin B12 as a co-factor. So, Vitamin B12 deficiency will lead to hyperhomocysteinemia [3].

Vitamin B12 is an essential micronutrient required for optimal haemopoetic, neuro-cognitive and cardiovascular function. Biochemical and clinical vitamin B12 deficiency has been demonstrated to be highly prevalent among patients with type 1 and type 2 diabetes

mellitus. The proposed mechanisms to explain metformin induced vitamin B12 deficiency among patients with T2DM include: alterations in small bowel motility which stimulates bacterial overgrowth and consequential vitamin B12 deficiency, competitive inhibition or inactivation of vitamin B12 absorption, alterations in intrinsic factor (IF) levels and interaction with the cubulin endocytic receptor. Metformin has also been shown to inhibit the calcium dependent absorption of the vitamin B12-IF complex at the terminal ileum [5].

Diabetic individuals are highly prone to coronary artery diseases (CAD) and hence it is necessary to search for advanced markers to assess the CAD risk. Increased plasma glucose leads to vascular dysfunction, therefore persons with diabetes have a two to four-fold greater risk of vascular disease occurrence as compared with persons with no diabetes. Studies have shown positive association between homocysteine and pathophysiology of diabetes mellitus. There are indications that elevated plasma homocysteine can predict early cardiovascular events. Hyperhomocysteinemia is defined as a medical condition characterized by an abnormally high level (above 15 $\mu\text{mol/L}$) of homocysteine in the blood [6].

This study is designed to assess the role of serum homocysteine and vitamin B12 in predicting early cardiovascular events in type 2 DM patients.

2. MATERIALS AND METHODS

- The present case control study was conducted at Dhiraj hospital, Piparia, Vadodara, Gujarat, India in which 80 subjects were enrolled, out of which 40

were cases of type 2 diabetes mellitus patients and 40 were controls.

2.1 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.

2.2 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.

2.3 Methods

- ❖ After obtaining the permission from ethical committee, data was collected by personal interview, with every individual under the guidance of the Mentor.
- ❖ Proforma was available for the filling of biodata such as age and gender, clinical examination findings, investigations like random blood glucose, glycated hemoglobin (HbA1c), serum homocysteine and vitamin B12. Participants were informed of the study purpose.
- ❖ We had taken 40 patients diagnosed with Type 2 Diabetes Mellitus as cases and 40 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 blood was collected in vacutainer tubes.
- ❖ Random blood glucose was estimated by glucose oxidase-peroxidase method and HbA1c by HPLC method in the Laboratory of Dhiraj hospital.
- ❖ Serum Homocysteine level was measured by Enzymatic Method [7] in the Laboratory of Dhiraj hospital.
- ❖ Serum Vit B12 level was measured by Chemiluminescence Immuno assay Method [8] in the Laboratory of Dhiraj Hospital.

2.4 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.

3. RESULTS

Results are presented as Mean \pm 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 random blood sugar was 282.95 ± 118.37 in cases and 105.02 ± 14.07 in controls. Patients of DM type 2 had significantly higher level of RBS 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 homocysteine was 14.15 ± 1.93 in cases and 7.27 ± 1.61 in controls. Patients of DM type 2 had significantly higher level of serum homocysteine 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 ($p < 0.0001$). Graph 1, 2, 3 shows graphical presentation of mean & SD levels of HCY, Vit B12 and HbA1c in cases and controls respectively. Table 2 shows correlation between HCY, Vit B12 and HbA1c. There is no correlation found between HCY, Vit B12 and HbA1c.

4. DISCUSSION

T2DM is a metabolic disease characterized by elevation of blood glucose concentrations, lipid abnormalities, and vascular complications. Diabetes is a major cause of both microvascular (retinopathy, nephropathy, and neuropathy) and

macrovascular diseases (cardiovascular diseases and nontraumatic lower extremity amputations), affecting, therefore, nearly every organ in the body [9]. Synthesis and metabolism of Hcy involves three processes: demethylation, remethylation and trans-sulfuration. The demethylation pathway is associated with creatinine generation, i.e., S-adenosylmethionine provides a methyl group with glycoamine on the generation of creatinine. Plasma homocysteine were found to be elevated in T2DM and also in pre- diabetes [10].

A study showed that an increase in serum Hcy level is an independent risk factor for early cardiovascular disease mortality in type 2

diabetes patients. Since homocysteine stimulates oxidative stress and inhibits nitric oxide formation, increased plasma homocysteine as observed in type 2 diabetic patients could promote platelet hyperactivity. It therefore suggests that some of the indicators of CVD risk factor associated with mortality in DM patients may be elevated plasma homocysteine and reduced folic acid and vitamin B12 [11]. Saurabh Bansal et al also showed increased levels of serum Hcy ($12.9 \pm 5.6 \mu\text{mol/L}$) in patients with type 2 diabetes mellitus as compared to that of non-diabetic individuals [12]. Our study results are coincides with the above studies. Patients of DM type 2 had significantly higher level of serum homocysteine than normal healthy individuals,

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

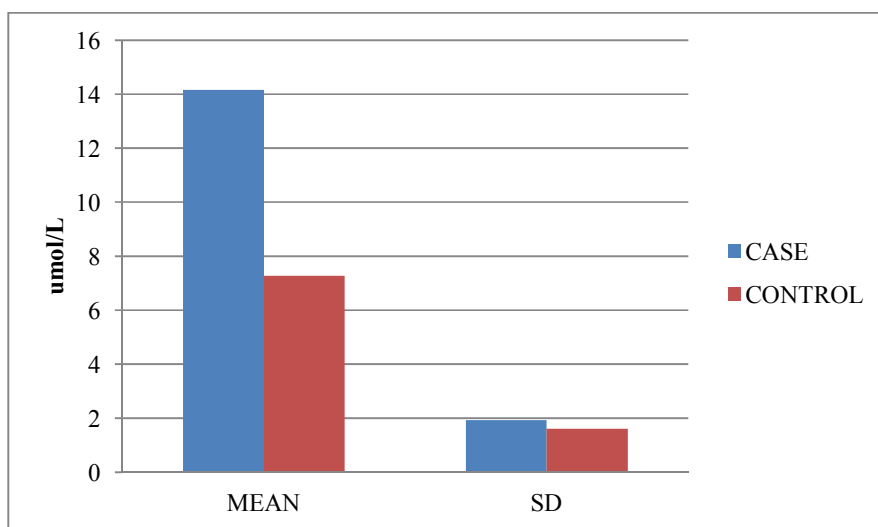
Parameters	Cases (n=40)	Controls (n = 40)	P value
AGE (years)	56.32 \pm 8.37	51.97 \pm 11.71	0.06
HbA1C (%)	9.50 \pm 1.69	5.17 \pm 0.53	<0.0001
RBS (mg/dl)	282.95 \pm 118.37	105.02 \pm 14.07	<0.0001
HCY(umol/L)	14.15 \pm 1.93	7.27 \pm 1.61	<0.0001
Vit B12(pg/ml)	149.87 \pm 37.69	547.85 \pm 105.17	<0.0001

DM – Diabetes Mellitus, RBS – Random blood sugar, HCY – Homocysteine, Vit B12- Vitamin B12; *p < 0.05 - significant, **p< 0.0001 - highly significant, #p \geq 0.05 - not significant

Table 2. Correlation between various parameters

Parameters	Cases		Controls	
	r value	P value	r value	P value
HCY and Vit B12	0.051	0.753	-0.169	0.296
HCY and HbA1c	0.060	0.708	-0.089	0.581
Vit B12 and HbA1c	0.006	0.967	-0.215	0.181

HCY – Homocysteine, Vit B12- Vitamin B12, HbA1c-Glycated Hemoglobin
*p < 0.05 - significant, **p< 0.0001 - highly significant, #p \geq 0.05 - not significant

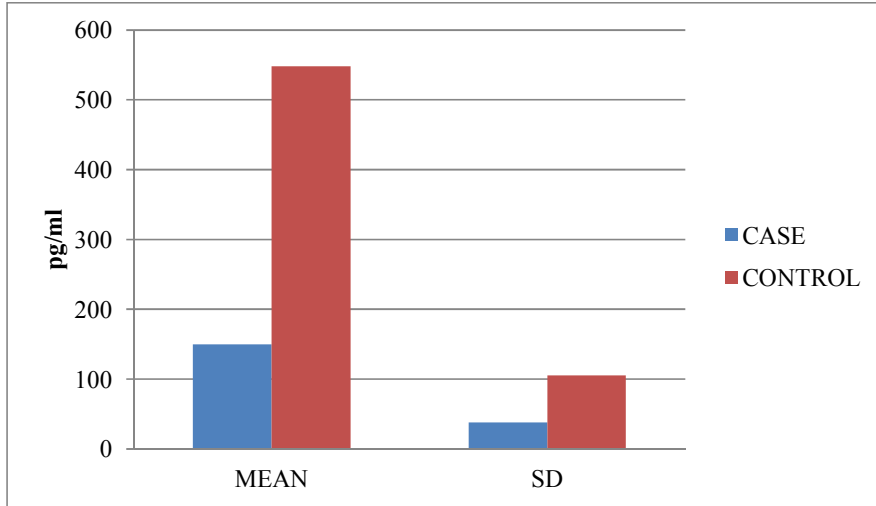


Graph 1. Shows mean & SD levels of s. HCY in cases and controls

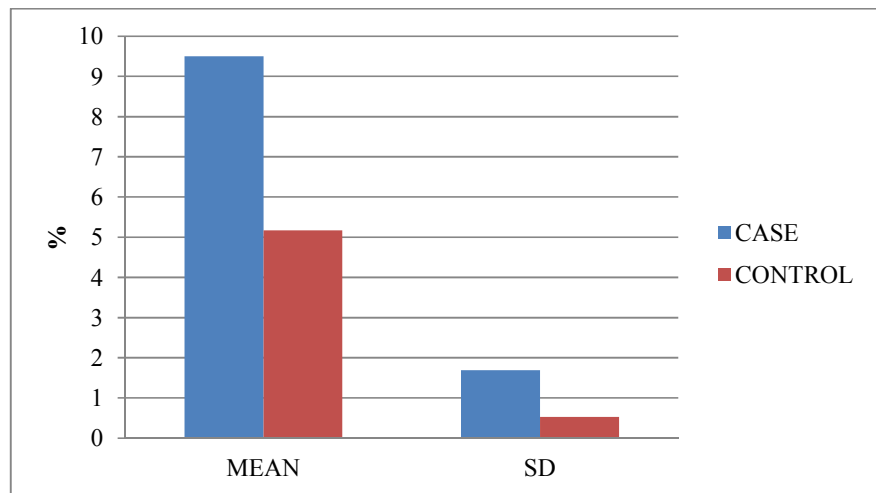
difference between them was statistically highly significant. The results of one study showed that Hcy levels were significantly reduced among subjects with Type 2 diabetes compared to those without diabetes. They observed a significantly negative correlation between hyperhomocysteinemia and T2DM. This negative correlation remained significant whether Hcy plasma levels were ≥ 10 or ≥ 15 , indicating a consistent threshold association. The reduction of Hcy levels among subjects with diabetes could possibly be attributed to two factors. First, Hcy is located at a branch-point of multiple metabolic pathways and is produced from methionine as a product of a large number of transmethylation reactions. Homocysteine methyltransferase (MTHFR) and cystathionine beta synthase (CBS) carry out a chemical reaction that converts Hcy

to methionine when Hcy is methylated by N-5 methyltetrahydrofolate. This remethylation reaction is the main regulator of plasma Hcy levels. Second, it was consistently shown that in rats with diabetes, the expression of CBS is significantly increased. Hcy levels were significantly increased when these rats with diabetes received insulin. These results strongly suggest a regulatory role of insulin in the hepatic trans-sulfuration pathway that metabolizes Hcy [13].

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 results coincides with the Cohort study done by Matthew C. Pflipsen et al. who also identifie



Graph 2. Shows mean & SD levels of vitamin B12 in cases and controls



Graph 3. Shows mean & SD levels of HbA1c in cases and controls

22% of type 2 diabetic patients with B12 deficiency [14]. The study done by Shailendra D et al showed that vitamin B12 deficiency was observed in about 10% of individuals with T2DM, which is mostly associated with metformin therapy of ≥ 2 g/day [15]. 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. This observation has been attributed to either or a combination of alteration in small bowel motility (which stimulates bacterial overgrowth with consequential vitamin B12 deficiency), alteration in intrinsic factor levels, interaction with the cubulin endocytic receptor and inhibition of the calcium dependent absorption of vitamin B12-intrinsic factor complex at the terminal ileum. Therefore, routine estimation of vitamin B12 level in patients with T2DM might help in identifying patients that would benefit from vitamin B12 supplements [16]. A study showed that mean levels of vitamin B12 decreased with increasing severity of glucose tolerance. Individuals with T2DM had the lowest values followed by those with prediabetes and normal glucose tolerance [17].

In the study done by L Ramachandran, they found that there was no significant difference between the patients of type 2 diabetes mellitus and the healthy control group with regard to plasma homocysteine levels. However, they found that homocysteine levels were significantly higher in complicated diabetes than diabetes without complications. They also found that the HbA1c has a positive correlation with homocysteine levels [18]. In our study we didn't find any correlation between HbA1c, homocysteine and Vitamin B12. But study done by Yogendra Narayanrao Keche et al showed that as higher homocysteine level in poorly controlled type 2 diabetic patient is associated with poor glycemic control and micro-macro vascular complication, correction of homocysteine level and correction of vitamin B12 deficiency which is one of the factor for raised homocysteine level would have resulted in beneficial improvement in HbA1c in their study [19]. Results obtained by our study are correlated with the study done by Maria Onomhagan Ebesunun et al. In their study, moderately raised plasma homocysteine was obtained with associated decreased vitamin B12 levels in type 2 diabetes subjects. The plasma Hcy obtained in their study did not correlate with vitamin B12 levels [20]. The result from our study has showed that increased plasma homocysteine, decreased

vitamin B12 could be used as possible predicting risk factors for premature cardiovascular events in type 2 DM.

5. CONCLUSION

A significant increase in serum Hcy level was observed in T2DM patients in our study. It has been shown in some studies that hyperhomocysteinemia is a risk factor for cardiovascular complications. T2DM patients are more prone for cardiovascular complications. Therefore, the hyperhomocysteinemia could serve as another important marker of poor diabetic control and developing complications. In this study, vitamin B12 deficiency was observed in T2DM patients. Oral vitamin B12 supplementation protects against vitamin B12 deficiency in patients with T2DM. Raised serum Homocysteine (s.Hcy) is considered as an early marker of B12 deficiency. Thus if we identify and treat B12 deficiency early, by measuring s. Hcy level, cardiovascular complications can be prevented or delayed.

CONSENT

As per international standard written patient consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical permission has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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