

Serum Homocysteine Levels in Type 2 Diabetes Mellitus Patients

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ABSTRACT

Introduction: Diabetes mellitus (DM) is one of the most frequently occurring endocrine disorders characterized by the common phenotype of hyperglycemia. 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. The current study was aimed to evaluate the status of atherogenic marker (serum Hcy) in type 2 diabetes mellitus patients and compare it with that of controls.

Material and Methods: This study was conducted on 30 patients of T2DM and 30 normal controls of corresponding age and sex. Fasting blood sample was taken from each patient and control and was analyzed for plasma glucose (GOD-POD method), HbA1c (Immunoturbidimetric method), serum Homocysteine (Ezymatic assay), serum Cholesterol (CHOD-PAP method), serum Triglycerides (GPO method) and serum HDL (Direct method) levels. Data was analyzed by using SPSS-20 version.

Results: In our study, the mean value of serum Hcy in diabetic patients was found to be 12.9 ± 5.6 $\mu\text{mol/L}$, which was significantly higher than that of the controls with the mean value of 9.9 ± 3.6 $\mu\text{mol/L}$ ($p > 0.05$). A significant positive correlation was also seen between serum Hcy levels and fasting plasma glucose with correlation coefficient of $r = 0.631$ ($p < 0.001$) and between serum Hcy level and HbA1c with $r = 0.416$ ($p < 0.05$).

Conclusion: Atherosclerotic vascular complications are responsible for majority of diabetes related morbidity and mortality. Serum Hcy independently can be used as predictor for cardiovascular risk events in T2DM patients.

Key words: Homocysteine, Diabetes Mellitus, Coronary Artery Disease (CAD).

INTRODUCTION

Diabetes mellitus (DM) is one of the most frequently occurring endocrine disorders characterized by the common phenotype of hyperglycemia. It is a growing health care problem worldwide and is characterized by metabolic abnormalities such as diabetic ketoacidosis and non-ketotic hyperosmolar coma and leads to complications involving the eyes, kidney, nerves, blood vessels and the gastrointestinal tract.¹

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. Diabetes Mellitus being a chronic disorder results from various factors in which a complete or partial deficiency or impaired function of insulin occurs. The most frequent grievous disorder that effects type 2 diabetic individuals is cardiovascular disease. Among diabetic subjects the risk for cardiovascular disease is 2-4 folds greater in comparison to normal subjects.²

Diabetes mellitus is one of the major risk factor for the progression of atherosclerosis, which is two to three folds more common as compared to that of normal population.

Elevated concentrations of serum Homocysteine (Hcy) is

expected to enhance the production of oxidation products such as Hcydisulfides and Hcythiolactone, leading to endothelial cell damage by extravagant sulfation of collagen which in turn aggravates the progression of thrombosis and arteriosclerosis. Plasma homocysteine levels are elevated in both viz; type 2 diabetic patients as well as in pre-diabetic individuals with insulin resistance. In such individuals, plasma Hcy concentrations is influenced by the insulin concentrations and anti-diabetic therapy such as metformin, glitazones or insulin that can either elevate or reduce the plasma Hcy concentrations.³ Hyperhomocysteinemia is increased in insulin resistant and hyperinsulinemic patients, and also in T2DM patients with intact pancreatic β -cell function.⁴ But when these patients lose pancreatic β -cells, they might then show a fall in plasma Hcy concentrations. Non-diabetic i.e. normal individuals who are having insulin-resistance syndrome also show higher plasma Hcy concentrations which proves the association between elevated plasma Hcy concentrations and increased plasma insulin concentrations.^{5,6}

As diabetic individuals are at higher risk of vascular disease, therefore it is required to look for reliable markers that can help in predicting prognosis and diagnosis of the disease. This study aims at finding a reliable correlation between serum homocysteine levels and insulin levels which shall indicate the degree of insulin resistance in patients with Type 2 Diabetes Mellitus, and hence predict their risk for atherosclerosis and cardiovascular disease.

MATERIALS AND METHODS

In the present study 30 patients aged 40 to 70 years who were diagnosed with type 2 diabetes were selected from the Medicine OPD and IPD of Teerthanker Mahaveer Medical College and Research Centre, Moradabad. 30 normal healthy subjects, age and sex matched with the diabetic patients, were selected as controls.

Exclusion Criterion: Patients having history of CAD, peripheral vascular disease, stroke, epilepsy, smoking, drug intake other than anti-diabetic drugs were excluded from the present study. Those individuals who were suffering from other inflammatory diseases like Tuberculosis, leprosy, cancer, skin diseases, Gout, Liver and kidney diseases and pregnancy were also excluded to rule out any increase in inflammatory markers due to other causes.

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Parameters	Diabetic Mean ± SD	Non-Diabetic Mean ± SD	t - value	p - value
FPG (mg/dl)	226 ± 126	88 ± 8	5.951	0.000
HbA1c (%)	11.5 ± 3.01	5.15 ± 1.06	9.416	0.000
Hcy (µmol/L)	12.9 ± 5.6	9.9 ± 3.6	1.544	0.128
TG (mg/dl)	143.7 ± 23.6	149.3 ± 38.2	0.688	0.494
Cholesterol (mg/dl)	182.6 ± 20.4	168.8 ± 21.4	2.533	0.014
HDL (mg/dl)	40.1 ± 3.1	47.5 ± 6.4	5.713	0.000
LDL (mg/dl)	113.8 ± 19.3	91.5 ± 20.2	4.362	0.000
VLDL (mg/dl)	28.73 ± 4.7	29.7 ± 7.6	0.688	0.494
TG/HDL	3.6 ± 0.6	3.2 ± 1.2	1.478	0.145
LDL/HDL	2.8 ± 0.5	1.9 ± 0.6	6.598	0.000

Table-1: Comparison of all parameters between study groups.

Correlation	r-value	p-value
Hcy and FPG	0.631	0.00
Hcy and HbA1c	0.416	0.022

Table-2: Correlation of Hcy with FPG and HbA1c

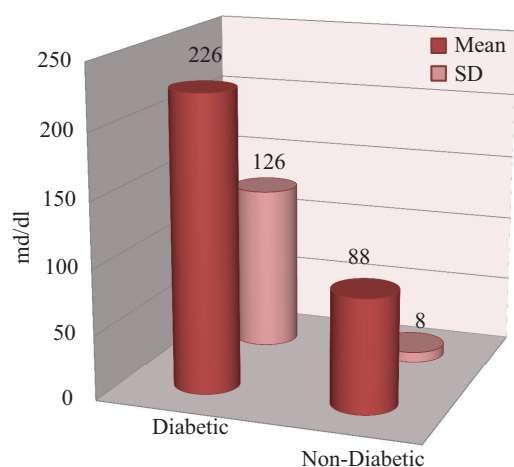


Figure-1: Comparison of FPG level between study groups

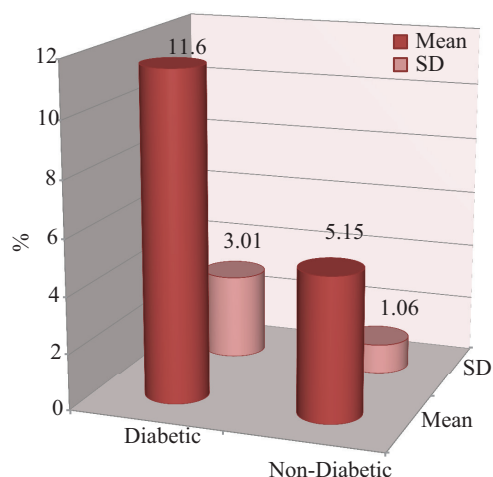


Figure-2: Comparison of HbA1c level between study groups

Investigations: All patients were subjected to following investigations:

- FPG GOD-POD Method⁷
- HbA1c Immunoturbidimetric Method⁸
- Hcy Enzymatic Assay^{9,10}
- Cholesterol CHOD-PAP Method^{11,12}
- Triglyceride GPO-Method¹³
- HDL Direct Method^{14,15}

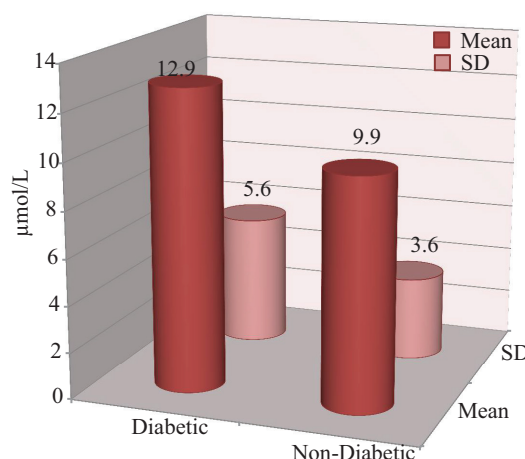


Figure-3: Comparison of Hcy level between study groups

Sample Collection: After overnight fasting for 8 hours, about 5 ml of venous blood was drawn with aseptic precaution from antecubital vein of all the subjects and dispensed into following vials for various biochemical tests:-

1. EDTA vial for glycosylated hemoglobin (HbA1c) estimation.
2. Fluoride oxalate vial for fasting plasma glucose (FPG) estimation.
3. Plain vial for Hcy, Creatinine and Lipid Profile.

Procedure: All the parameters viz. FPG, HbA1c, Hcy, Cholesterol, Triglyceride and HDL were measured on ERBA Mannheim EM200 automatic analyser.

STATISTICAL ANALYSIS

Mean ± SD were calculated for all the parameters analyzed and were compared by Student's t-test. The parameters were correlated by the calculation of coefficient of correlation (r-value) using SPSS program. p-values; p <0.05 and p <0.001 were considered significant highly significant respectively.

RESULTS

In the present study the mean level of HbA1c and Serum FPG, Cholesterol, HDL, LDL, and LDL/HDL were significantly higher in the diabetic group as compared to the control group. Whereas, Hcy and TG/HDL although apparently higher in diabetic group showed no significant variation from the control group (table-1).

In the present study, a significant positive correlation was seen between serum Homocysteine level with fasting plasma glucose and serum Homocysteine level with HbA1c (table-2,

figures-1,2,3).

DISCUSSION

Diabetes Mellitus (DM) consists of a set of metabolic disorders that share a common phenotype of hyperglycemia. Several different types of diabetes mellitus exist having different etiological factors such as genetic, environmental and life style changes. Depending on the etiology, various factors may influence hyperglycemia through various mechanisms which may include either low insulin secretion and diminished glucose usage or increased glucose production. The metabolic dysregulation in diabetic individuals leads to secondary pathophysiological changes in multi-organ systems which in turn aggravates the heavy burden on the diabetic individuals and on the health care system.¹⁶ Nickolas et al¹⁷ has stated that atherosclerosis is a chronic low-grade inflammatory disease in diabetes mellitus. The marker included in the present study - serum Homocysteine (Hcy) can be used as predictors for atherogenesis leading to CAD.

The present study shows 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, ($9.9 \pm 3.6 \mu\text{mol/L}$).

Prashanth et al in his study concluded that serum Hcy levels in T2DM patients are associated with pre-hypertension which increases cardiovascular risk.¹⁸ In a community based study of Chinese population, Feng et al¹⁹ found that alterations of aortic stiffness is associated with serum Hcy levels. Ebru et al also observed in the study that serum Hcy is independent risk factors for cardiovascular disease.²⁰ All the above mentioned studies and researches had a close match with our study which signifies that serum Hcy can be used as predictor for atherosclerosis.

Serum Homocysteine (Hcy) and T2DM: Numerous studies have shown altered serum Hcy concentrations in T2DM patients. The outcome of earlier studies are variable but many of them have shown increased serum Hcy levels in T2DM patients. Hyperhomocysteinemia is influenced by insulin resistant and hyperinsulinemic patients, and also in T2DM patients with intact pancreatic β -cell function.⁴ But when these patients lose pancreatic β -cells, they might then show a fall in plasma Hcy concentrations. Non-diabetic i.e. normal individuals who are having insulin-resistance syndrome also show higher plasma Hcy concentrations which proves the association between elevated plasma Hcy concentrations and increased plasma insulin concentrations.^{5,6}

In our study the mean value of serum Hcy in diabetic patients was found to be $12.9 \pm 5.6 \mu\text{mol/L}$, which was higher than that of the controls with the mean value of $9.9 \pm 3.6 \mu\text{mol/L}$ ($p > 0.05$).

Hcy and Glycaemic Status: On comparing the serum Homocysteine levels with the metabolic control of diabetes (i.e. HbA1c) our findings were significant indicating ($p < 0.05$) that serum Hcy levels increases significantly with poor long term glycaemic control (HbA1c $> 7.0\%$). Our findings go with the study conducted by Drzewoski et al⁴ who found that among the diabetes patients there was highly significant correlation ($p < 0.05$) between serum Hcy and HbA1c, while no significant difference ($p > 0.05$) was found by Araki et al [100]. We also found a positive higher significance between serum Hcy and FPG with a correlation coefficient of $r = 0.631$ ($p < 0.001$).

Hcy and Lipid Profile: In the present study, although significantly higher values of serum Cholesterol, LDL, and HDL were found but no significant correlation could be established between these parameters and serum Homocysteine.

Similarly, significant difference was found by Balu et al²¹ between serum Hcy levels (15.064 ± 4.82 , $p < 0.001$), serum Cholesterol (201.2 ± 29.69 , $p < 0.001$), LDL (128.08 ± 29.09 , $p < 0.001$) and HDL (40.08 ± 3.29 , $p < 0.05$) in comparison to that of controls.

Chico et al²² in their study observed relatively high mean value of serum Hcy levels in T2DM with 'p' value < 0.05 . On the contrary, Wollesen et al observed the insignificant levels ($p > 0.05$) of serum Hcy in type 2 diabetic patients ($10.6 \mu\text{mol/L}$) as compared to that of control subjects ($11.1 \mu\text{mol/L}$).²³

It has been proposed that increased levels of serum Hcy levels leads to endothelial impairment by enhancing oxidative stress.²⁴ and declines the release of nitric oxide (NO), which impairs vascular dilatation.²⁵ Hyperhomocysteinemia stimulates proliferation of smooth muscle cell and synthesis of collagen which in turn promotes intima-media thickening.^{26,27} High serum Hcy levels considered to have thrombogenic activity by altering coagulation system and stimulate on of platelet aggregation. Excess of serum Hcy level is also seen to be related with enhanced lipid peroxidation which also predisposes to atherosclerosis.²⁸

Balu et al in their prospective study has found a potent and independent association between Hyperhomocysteinemia and lipid profile in T2DM which leads to coronary heart diseases. They observed that serum Hcy levels were significantly higher in T2DM ($15.1 \pm 4.7 \mu\text{mol/L}$), than in control group ($8.9 \pm 2.96 \mu\text{mol/L}$).²¹

Lentz in the study concluded that serum Hcy levels are an independent risk factor for cardiovascular disorders in T2DM patients.²⁹ Hoogeveen et al stated that increased levels of serum Hcy is by 1.6 times stronger cardiovascular disease risk factor in type 2 diabetes mellitus patients than in non-diabetic individuals.³⁰

These studies suggest that Hyperhomocysteinemia may have both atherogenic and thrombogenic effects predisposing to adverse cardiovascular consequences.

In this study, the values of serum Hcy were relatively higher in diabetic cases in comparison to non-diabetic cases. It suggests that serum Hcy may serve as an atherogenic and thrombogenic marker and can be used as a predictor of cardiovascular risk in T2DM patients.

CONCLUSION

Low-grade Inflammation is considered to be an important aspect of metabolic syndrome. Atherosclerotic vascular complications are responsible for majority of diabetes related morbidity and mortality.

A significant positive correlation between serum Hcy with long and short term glycaemic status was observed in our study which hypothesizes that the association between hyperhomocysteinemia and increased rate of coronary heart disease events is significant in the diabetic patients.

Therefore, it can be concluded that serum homocysteine can be used as a predictor for cardiovascular risk events in T2DM patients.

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