ORIGINAL RESEARCH

Homocysteine level and its correlation with lipid profile and glycemic parameters in type 2 diabetes mellitus patients

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ABSTRACT

Background: Diabetes mellitus is a metabolic disorder arises due to defect in insulin secretion, insulin action or both. This disease causes many complications which includes cardiovascular diseases. There are many causes of CVD like raised homocysteine level. Thus, this study was conducted to find out the homocysteine and blood sugar level in type 2 D.M. patients in relation to CVD risk factors. **Materials and Methods:** 300 Subjects were taken for the study in which 200 were diagnosed diabetes cases and 100 were healthy control subjects. Blood collected from subjects was centrifuged for estimation of serum homocysteine, vitamin B_{12}, fasting blood sugar (FBS), total cholesterol (TC), triglyceride (TG), high density-lipoprotein cholesterol (HDL-c) was estimated by Vitros 5600 Integrated Chemistry Analyzer based on "Dry Slides Techniques. Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were estimated by Friedwald's formula: LDL-c = TG- (HDL-c + VLDL-c) and VLDL-c = TG/5. **Result:** The homocysteine, glycemic parameters (FBS, PPBS, HbA1c) and lipid profile (Total cholesterol, triglycerides and VLDL-cholesterol) were significantly higher in T2DM patients at levels as compared to control subjects. Vitamin B_{12} was significantly lower in T2DM patients at 0.05 levels as compared to control subjects. Homocysteine is significant positively correlated (p-value<0.01) with HbA1c, TG and VLDL-cholesterol and significant negatively correlated (p- value<0.01) with HDL-cholesterol in T2DM patients. **Conclusion:** the genetic polymorphism may be the reason for increased homocystein level which causes insulin resistance and insulin resistance may be the reason for altered lipid profile.

Key words: Diabetes mellitus, homocystein, lipid profile, insulin resistance

INTRODUCTION

Diabetes mellitus is a metabolic disorder arises due to defect in insulin secretion, insulin action or both [1, 2]. This disease causes many complications which includes cardiovascular diseases (CVD), nerve damage, kidney damage etc [3]. There are many causes of CVD like raised homocysteine level [4] which is now regarded as new bad cholesterol. High level of homocysteine in the serum, above 15 µmol/L is a medical condition called hyper homocysteinemia [5, 6] which is associated with endothelial dysfunction in type 2 diabetes [7, 8]. Now, it is also a marker of endothelial cell damage [9, 10].

Homocysteine is responsible for generating reactive

oxygen species (ROS) and ROS is responsible for endothelial dysfunction of blood vessels [11-13]. It is also responsible for accumulation of lipid in endothelial which increased the risk of coronary heart disease especially in type 2 diabetes mellitus [14, 15]. Increased circulating level of homocysteine is seen in patients with homocystinuria or individuals with dietary deficiency of folate and or cynocobalamin [16, 17]. Type 2 diabetes mellitus are now considered to be a strong coronary heart disease factor because it affects several lipid metabolic pathways [18, 19]. Thus, this study was conducted to find out the homocysteine and blood sugar level in type 2 D.M. patients in relation to CVD risk factors.

MATERIALS AND METHODS

Study Design: The study was conducted in the department of biochemistry, G.R. Medical College, Gwalior (M.P.) and in the department of biochemistry, Clinical Laboratory, Medanta – the Medicity, Gurgaon, India. Institutional Ethical Committee, G.R. Medical College, Gwalior had approved this study.

Study Population: 300 Subjects were taken for the study in which 200 were diagnosed diabetes cases and 100 were healthy control subjects.

Study Record: Patients clinical histories were recorded in study proforma and consent was taken from every patient.

Inclusion Criteria: Diagnosed T2DM patients having fasting blood glucose ≥ 126mg/dl on 2 occasions.

Exclusion Criteria: Patients taking drugs which disturbed lipid and carbohydrates metabolism, renal disease patients and pregnant women were excluded from the study.

Sample Collection and Preparation: Blood samples were taken from the antecubital vein in plain vacutainers, fluoride vacutainers and EDTA vacutainers. Samples were centrifuged at 3000 rpm for 10 to 20 minutes. Supernatant collected after centrifuged at 3000 rpm for 10 to 20 minutes in sterile test tube for analysis of biochemical test.

Analysis of Sample: The serum homocysteine, vitamin B_{12}, fasting blood sugar (FBS), total cholesterol (TC), triglyceride (TG), high density-lipoprotein cholesterol (HDL-c) was estimated by Vitros 5600 Integrated Chemistry Analyzer based on "Dry Slides Techniques. Low density lipoprotein cholesterol (LDL-c) and very low density lipoprotein cholesterol (VLDL-c) were estimated by Friedwald's formula: LDL-c = TG- (HDL-c + VLDL-c) and VLDL-c = TG/5.

Statistical Analysis: The data was prepared in Microsoft Excel 2007. Statistical analysis was done in SPSS-23. Comparison of biochemical parameters of T2DM and control cases was done by Z test. Correlation of biochemical parameters were done by pearson correlation. Graph pad prism 7 was used for plotting the graph. P-value less than 0.05 were considered significant.

RESULTS

Mean and standard deviations (SD) of T2DM and control subjects were shown in table 2. The homocysteine, glycemic parameters (FBS, PPBS, HbA1c) and lipid profile (Total cholesterol, triglycerides and VLDL-cholesterol) were significantly higher in T2DM patients at 0.01 levels as compared to control subjects. Vitamin B_{12} was significantly lower in T2DM patients at 0.05 levels as compared to control subjects. Table 2 shows the correlation of homocysteine with lipid profile and glycemic parameters in T2DM patients. Homocysteine is significant positively correlated (p-value<0.01) with HbA1c, TG and VLDL-cholesterol and significant negatively correlated (p- value<0.01) with HDL-cholesterol in T2DM patients.

Table 1: Showing the significant changes of homocysteine, glycemic parameters and lipidprofile in type 2 diabetes mellitus patients and control subjects.

Parameters	Mean ± SD (Control)	Mean ± SD (Patients)	P-value
Homocysteine (µmol/L)	11.69 ± 6.71	14.67 ± 5.99	0.01**
Vitamin B_{12} (ng/mL)	451.82 ± 272.61	385.22 ± 216.0	0.03*
Fasting blood sugar (mg/dl)	89.70 ± 7.57	176.03 ± 57.12	0.00**
Glycosylated Hemoglobin (%)	5.42 ± 0.44	8.24 ± 1.77	0.00**
Post Prandial blood sugar (mg/dl)	116.98 ± 10.07	232.65 ± 85	0.00**
Total Cholesterol (mg/dl)	176.61 ± 39.06	189.03 ± 41.94	0.01**
Triglycerides (mg/dl)	130.36 ± 63.83	173.22 ± 100.96	0.00**
HDL-Cholesterol (mg/dl)	44.81 ± 12.14	41.98 ± 11.54	0.06^{NS}
LDL-Cholesterol (mg/dl)	105.73 ± 33.04	112.41 ± 44.88	0.11^{NS}
VLDL-Cholesterol (mg/dl)	26.07 ± 12.77	34.64 ± 20.19	0.00**

* Significant at 0.05 (p<0.05)
** Significant at 0.01 (p<0.01)
^{NS} Non-significant

Table 2: Showing the correlation of homocysteine with lipid profile and glycemic parametersin T2DM patients

Parameters	Homocysteine
Fasting blood sugar (FBS)	r = 0.112
Glycosylated Hemoglobin (HbA1c)	r = 0.417**
Post-prandial blood sugar (PPBS)	r = 0.009
Total Cholesterol (TC)	r = 0.030
Triglycerides (TG)	r = 0.323**
VLDL-cholesterol	r = 0.323**
HDL-cholesterol	r = -0.373**
LDL-cholesterol	r = -0.023
** Correlation is highly significant at the 0.01 level. NSNon-significant	

DISCUSSION AND CONCLUSION

Diabetes is a hazardous disease affecting all the metabolic pathways the body [20]. Homocysteine is a sulfur containing amino acid [21] which is formed from the metabolism of methionine [22]. Altered level of homocysteine had been found to be associated with many complications like cardiovascular diseases (CVD) in T2DM [22, 23]. In our study homocysteine, glycemic parameters (FBS, PPBS, HbA1c) and lipid profile (Total cholesterol, triglycerides and VLDL-cholesterol) were increased in T2DM patients as compared to control subjects. These results were supported by many researchers [24-26]. The level of homocysteine was increased in diabetic patients (14.67 ± 5.99) at p-value of 0.01. Many researchers have been conducted on homocysteine level in diabetic patients with variable finding [26, 27]. The possible mechanism of increased homocystein is that 5,10 methylene-tetrahydrofolate reductase (MTHFR)is the rate-limiting enzyme for the conversion of dietary folate to 5-methyltetrahydrofolate, the methyl group donor required for the remethylation of homocysteine to methionine in vivo. Hyperhomocysteinemia may be due to the presence of a thermo labile isoform of this key enzyme. A single base pair (677C > T) substitution in the human MTHFR gene predicts phenotypic expression of a heat-sensitive variant with reduced enzymatic activity. Elevated homocystein level caused by MTHFR genetic variants has been demonstrated to be associated with insulin resistance [28-32]. Insulin resistance may escort the overproduction of very low-density lipoprotein (VLDL) cholesterol. This leads to more triglyceride-rich particles, fewer HDL particles, and smaller, dense LDL [33]. On the basis of our finding we concluded that the genetic polymorphism may be the reason for increased homocystein level which causes insulin resistance and insulin resistance may be the reason for altered lipid profile.

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