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Serum homocysteine and its association with various biochemical parameters in type 2 Diabetes Mellitus

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Abstract

Aim: Homocysteine is a marker of endothelial dysfunction, which is suggestive of cardiovascular complications. In this study, we aimed to assess the role of homocysteine in the development of cardiovascular complications in type 2 diabetes.

Patients and methods: Total 100 Patients were recruited in this study following the exclusion and inclusion criteria.. 50 test subjects, diagnosed with type 2 Diabetes according to the American Diabetes Association (ADA) revised criteria 2016 and 50 healthy controls of both gender and age comparable to test group, were selected. Plasma blood sugar in fasting and postprandial sample, HbA1C and serum lipid profile were estimated by colorimetric method on the fully automated analyzer. Serum Hcy was done by chemiluminescence method.

Results: Diabetic subjects had significantly increased serum homocysteine, serum cholesterol, serum triglycerides (TG) and serum low density lipoprotein (LDL) ($p<0.001$). High density lipoprotein (HDL) was statistical insignificant and slightly lower in subjects with diabetes. Serum low density lipoprotein (LDL) ($p<0.001$) levels were significantly higher in the subjects having Homocysteine levels > 15 µmol/l.

Conclusions: Homocysteine has an important role in the development of cardiovascular complication and significantly increases levels of Homocysteine in diabetic subjects suggests that this marker can be used for early identification of diabetic patients at risk of developing such complications.

Keywords: Homocysteine, type -2 diabetes, low density lipoprotein, cardiovascular complications.

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. Its global prevalence was about 8% in 2011 and it's predicted to rise to 10% by 2030 [2]. According to the Indian Heart Association, India is the "Diabetic capital of the world" with a projected 109 million individuals with diabetes by 2035[3].

Homocysteine (Hcy), sulphur containing nonessential amino acid formed from demethylation of methionine has been implicated as an important risk factor for

atherosclerosis and arteriosclerosis. Patients with hyperhomocysteinemia and homocyteinuria display early onset of atherosclerosis and manifest arterial and venous thrombosis [4]. It has been suggested that homocysteine could be an important and independent predictor of complications, in diabetes mellitus especially atherothrombotic events [5].

Glycated hemoglobin (HbA_{1C}) is a routinely used marker for long term glycemic control. HbA_{1C} predicts the risk for the development of diabetic complications in diabetes mellitus [6]. Apart from classical risk factors like dyslipidaemia elevated HbA_{1C} has now been regarded as an independent risk factor for cardiovascular disease in subjects with or without diabetes. The estimated risk of cardiovascular disease (CVD) has shown to be increased by 18% for each 1% increase in absolute HbA_{1C} value in diabetic population [7].

Studies have shown a positive correlation between glucose intolerance and cardiovascular disease with obesity, dyslipidaemia, hypertension, polycystic ovary disease, smoking, sedentary lifestyle; certain ethnic groups, poorly regulated diabetes, and hyperinsulinemia due to any reason or risk factors. However, not all of these factors were able to explain the strong association of diabetes with premature atherosclerosis. Therefore the present study was undertaken to evaluate the association of Hcy with other biochemical parameters in type 2 diabetes mellitus and to identify the possible risk factors associated with CVD.

Materials and methods

The study was conducted on 100 patients with type 2 diabetes mellitus attending the Department of Medicine. Type 2 diabetes was diagnosed according to the American Diabetes Association (ADA) revised criteria 2016 [8].

50 healthy subjects were selected of both genders and age comparable to test group, detailed history, clinical examination and relevant investigations were conducted to exclude control suffering from such diseases which is likely to affect serum lipid profile, blood sugar, serum homocysteine level, like hypertension, coronary artery disease, liver diseases hyper and hypothyroidism, various chronic and acute infections, pregnancy etc.

Plasma blood sugar in fasting and postprandial sample, HbA_{1C} and serum lipid profile were estimated by colorimetric method on the fully automated analyzer. Serum Hcy was done by chemiluminescence method.

All values are expressed as mean values with standard deviation (SD). The observation was compared with student's t test and p values <0.05 were considered to indicate statistical significance.

Results and discussion

Table 1 represents the biochemical characteristics of control group and diabetic group. The diabetic subjects had significantly ($p< 0.001$) higher blood sugar as compared with the control group. Further, we observed that diabetic subjects had significantly increased serum homocysteine, serum cholesterol, serum triglycerides (TG) and serum low density lipoprotein (LDL) ($p<0.001$). High density lipoprotein (HDL) was statistical insignificant and slightly lower in subjects with diabetes.

Table – 1: Biochemical parameters in control and diabetic group.

Variables	Control subjects	Diabetic subjects	p - value
Blood sugar (mg%)	99.14 ± 12.58	245.6 ± 69.2	<0.001
Homocysteine (µmol/l)	10.19 ± 3.07	20.15 ±7.13	<0.001
Cholesterol (mg/dl)	151.24 ±28.23	172.5 ±43.8	<0.001
Triglycerides (mg/dl)	115.32 ± 42.75	147.0 ± 68.6	<0.001
HDL (mg/dl)	45.64 ± 6.93	42.0 ± 9.20	NS
VLDL (mg/dl)	23.06 ± 8.55	29.39 ± 13.72	NS
LDL (mg/dl)	82.54 ± 21.70	101.0 ± 31.7	<0.001

HDL- High density lipoprotein

LDL- low density lipoprotein

VLDL – Very low density lipoprotein

NS- Not significant

Table 2 shows the distribution of biochemical parameters according to the patient's homocysteine level. In the present study diabetic group (n=100) was divided into two categories. It was observed that 27 patients of type 2 diabetes mellitus had Hcy levels ≤ 150 µmol/l and 73 had Hcy levels > 15.0 µmol/l. The mean blood glucose in the two subgroups were 245 ± 73.10 mg% and 245.8 ± 68.16 mg% respectively. In the subgroup with Hcy > 15.0 µmol/l, higher levels of serum cholesterol, serum triglycerides, serum LDL and serum HDL were observed as compared to that with Hcy levels ≤15. 0 µmol/l subgroup.

Table- 2: Distribution of parameters according to the lipid profile categorised by patient's Hcy levels.

Parameters	Hcy ≤ 15.0	Hcy >15.0	p-value
Blood sugar (mg%)	246.0 ± 73.10	245.9 ± 68.16	NS
Homocysteine (µmol/l)	12.53 ± 1.12	23.0 ± 6.3	<0.001
Cholesterol (mg/dl)	151.26 ± 36.64	180.0 ±43.90	0.003
Triglycerides (mg/dl)	136.74 ± 58.47	151.0 ± 72.0	NS
HDL (mg/dl)	40.25 ± 8.77	42.58 ± 9.42	NS
VLDL (mg/dl)	27.35 ± 11.69	30.14 ± 14.39	NS
LDL (mg/dl)	83.65 ± 28.91	107.6 ± 30.42	<0.001

HDL- High density lipoprotein

LDL- low density lipoprotein

VLDL – Very low density lipoprotein

NS- Not significant

Further, in the present study out of 100 diabetic subjects, 59 patients were advised HbA_{1C} levels by the treating physician. Out of 59 patients whose HbA_{1C} levels were estimated, 22 patients had HbA_{1C} ≤ 8.0% and 37 patients had >8.0%. Hypercholesteremia was found in those who had HbA_{1C} >8.0%. Similarly hypertriglyceridemia and increased LDL-C was found. Decreased HDL-C was found in 37 patients who had HbA_{1C} >8.0%. A significant association was observed between triglycerides and HbA_{1C} levels. The mean level of TG was higher in the group with HbA_{1C} >8.0% (P= 0.017). However, serum cholesterol, HDL-C and LDL –C did not show a significant association with HbA_{1C} (Table 3).

Table -3 Biochemical parameters categorised by patient's HbA_{1C} level.

Parameters	HbA_{1C} ≤ 8%	HbA_{1C} >8%	p - value
HbA_{1C} (%)	6.93 ± 0.79	10.19 ± 1.59	<0.001
Homocysteine (µmol/l)	16.95 ± 6.05	21.24 ± 8.16	0.037
Cholesterol (mg/dl)	163.82 ± 43.54	167.65 ± 47.27	NS
Triglycerides (mg/dl)	131.59 ± 46.96	167.65 ± 58.19	0.017
HDL (mg/dl)	40.95 ± 8.28	39.16 ± 7.94	NS
LDL (mg/dl)	95.27 ± 36.64	96.82 ± 33.64	NS

The present study was conducted on 100 patients suffering from diabetes mellitus and 50 normal subjects. The control subjects included in this study were asymptomatic persons free from any abnormality on routine examination, not taking any drug, not on vitamin supplements and were free from diseases.

In the present study, we observed that there is a significant difference of serum homocysteine, serum cholesterol, and serum LDL -C in the control group and type 2 diabetic group. This Study is strongly in agreement with Shiderman AD et al., 2001 [9]. They described the unappreciated atherogenic dyslipoproteinemia in type 2 diabetes mellitus. Hypertriglyceridemia and increased numbers of small dense low-density lipoprotein particles and low levels of high-density lipoprotein cholesterol were reported in their study.

Elevated plasma Hcy concentration is considered as an independent risk factor for atherosclerosis in subjects with normal glucose tolerance. Although type 2 diabetes is definitely associated with premature atherosclerosis; only a few studies have dealt with the association between hyperhomocyteinemia and micro or macro angiopathy complications with contradictory results.

Hyperhomocysteinemia has been reported in type 2 diabetes mellitus in several studies [10-12] and may contribute to the development of vascular complications [13]. Several studies have shown that higher levels of Hcy in the blood are related to a higher risk of coronary heart disease, stroke, and peripheral vascular disease [14-18].

Puri A et al. (2003) reported that the mean levels of Hcy in patients (27.80 ± 13.11) µmol/l were almost twice that of the controls (13.22 ± 7.36) µmol/l; while 72.55% patients had Hcy levels greater than 18 µmol/l, only 26.67% controls had increased levels [19]. These findings are similar to the observations of the present study in which 73% of the patients in diabetic group had Hcy level > 15.0 µmol/l. In the above mentioned study there was no significant difference, in the Hcy levels, in relation to the lipid profile. Further, they found hyperlipidemia was seen in 58.82% of the patients in their study. Elevated serum cholesterol is causally associated with increased risk of CAD. Specifically 10% increase in serum cholesterol is associated with a 20 to 30% increased risk of CAD and elevations earlier in life may be associated with higher risk of CAD as reported by La Rosa et al. 1990 [20].

Our study reveals high prevalence of hypercholesterolemia, high LDL-C and low HDL-C levels which are well known risk factors for CVD. Insulin affects the liver apolipoprotein production. It regulates the enzymatic activity of lipoprotein lipase and cholesterolester transport protein. All these factors are likely cause of dyslipidaemia in diabetes mellitus [21]. The main disorder in the present study is lipid metabolism, was hypertriglyceridemia. The finding is in concordance with the previous study by Regmi P et al., (2009) [22].

Further, we observed the impact of glycemic control on various lipid parameters. Severity of dyslipidaemia increases in patients with higher $HbA_{1C.}$ As elevated HbA_{1C} and dyslipidaemia are independent risk factors of CVD, diabetic patients with elevated HbA_{1C} and dyslipidaemia can be considered as a very high risk group of CVD.

Pouwels M et al., (2003) investigated effect of improved insulin sensitivity and glycemic control on plasma homocysteine [23]. They confirmed that the glycemic control did not influence plasma homocysteine level [23, 24]; On the other hand, there are some studies which suggest that glycemic control of diabetes may influence Hcy levels [25, 26]. These studies proposed that serum homocysteine level might be one of the factors underlying the link between hyperglycemia and cardiovascular risk indiabetic patients [25]. These authors have shown that patients with poor glycemic control of diabetes had significantly higher Hcy in comparison to diabetes with normal HbA_{1C} levels.

The overall findings of the present study suggest that type 2 diabetic patients are at a risk of developing hyperhomocysteinemia as well as dyslipidaemia. While homocysteine is a marker of endothelial dysfunction, dyslipidaemia is suggestive of cardiovascular complications . Assessment of these risk markers can therefore, be helpful in early identification of patients at risk of developing such complications.

Conclusion

Homocysteine, serum cholesterol, serum triglycerides (TG) and serum low density lipoprotein (LDL) are suggestive of dyslipidemia which can further lead to cardiovascular complications in Diabetic subjects. This alludes the idea that the assessment of these risk markers

can therefore, be helpful in early identification of patients at risk of developing such complications.

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