INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

HOMOCYSTEINE A NOVEL MARKER OF CORONARY ARTERY DISEASE.

Biochemistry

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ABSTRACT

Hyperhomocysteinemia has recently been identified as a risk factor for coronary artery disease(CAD). This study hence is an attempt to determine the association of homocysteine (t hcy) with severity of CAD in patients with and without type 2 DM and their correlation with insulin resistance and lipid profile.

KEYWORDS

Hyperhomocysteinemia, CAD, insulin resistance.

INTRODUCTION

Coronary artery disease (CAD) is a major cause of death and impairment of quality of life in patients with type 2 diabetes mellitus (T2DM) (1). Its early detection and rapid intervention is critical in the management of these patients. However in patients with T2DM, CAD generally is detected at an advanced stage, whereas an asymptomatic stage is often missed (2). More over in half of those who develop CVD, the established risk factors are found to be absent .Thus cardiovascular risk assessment based on conventional risk factors does not fully explain the distribution of risk (3). Since conventional coronary risk factors do not directly reflect structural and functional changes in arteries caused by atherosclerosis that is closely associated with CAD, additional factors are necessary to identify the people who are at risk of CVD (4).Over the past decade, identification of novel risk factors and predictors for CVD has been an area of major interest in preventive cardiology.An increase in total plasma homocysteine (hcy) is considered to be one of these new factors (5).

MATERIALS AND METHOD

The study was conducted at the department of biochemistry and cardiology .Study group consisted of 109 individuals with established CAD in the age group of 40-70 years who had undergone coronary angiography and diagnosed with CAD including single vessel, double vessel and triple vessel and 71 healthy individuals matched for age and sex.

The subjects were grouped into CAD patients with type 2 DM(CADWDM) (n=57)and CAD patients without type 2 DM (CADWNDM)(n=52) and normal healthy group(n=71).From each patient, their medical history was obtained through a structured questionnaire and an informed consent was obtained. The study was approved by the Institutional Ethics Committee.

The inclusion criteria include patients with established CAD including single vessel, double vessel and triple vessel. Those who had undergone a treadmill test positive for inducible ischemia, patients with history of essential hypertension, CAD patients with essential hypertension who had border line rise in fasting blood glucose, and patients with recent onset of diabetes.T2DM was diagnosed according to the WHO criteria (6).

Patients excluded were those diagnosed to have CAD with atrial fibrillation or pacemaker, history of congestive heart failure, history of stroke, transient ischemia or carotid surgery, history of coronary artery bypass graft surgery or percutaneous, transluminal coronary angioplasty, history of intermittent claudication or peripheral vascular surgery. Venous blood sample was collected after an overnight fast of 12 hours and the serum was used for the estimation of fasting blood glucose (FBG) by enzymatic GOD-POD method , cholesterol by enzymatic 'CHOP-PAP' method , triglyceride(TG)by enzymatic GPO-POD method and high density lipoprotein cholesterol (HDL-C) by direct enzymatic colorimetric method LDL-C and VLDL-C were calculated using the Friedewald's formula .Homocysteine was measured using an enzymatic assay(7).

Data are expressed as mean ± SD. Independent t-test was used to compare means of the various variables between controls and CAD subjects. Pearson correlation was used to see the correlation of various variables with hcy.

RESULTS AND DISCUSSION

Among the study subjects, the control subjects 65% were males and 35% were female Among the CAD WNDM patients 88% were males and 12% were female and in CAD WDM 82% were male and 18% were female .The base line characteristics of study subjects are shown in (Table 1). The mean age of onset of CAD in the group with type 2 DM was 52 ± 4.5 when compared to 59± 6.4 in CAD WNDM. The mean duration of diabetes in CAD WDM was 6.2±2.5. Statistically no significant difference in SBP and DBP was observed in CAD WDM when compared to CAD WNDM. Significant difference in BMI was observed in the CAD WDM subjects when compared to CAD WNDM and control (with p<0.001).

Table 1 Demographic details of study subjects.

PARAMETERS	CONTROL (N=71)	CAD WNDM(N=52)	CAD WDM(N=57)
Age(Years)	52.42 ±6.7	55.4 ± 5.657	52 ±9.5
BMI (kg/m2)	20.05±0.95	27.82 ± 3.359a	32.05 ± 0.33a,b
SBP (mmHg)	116.14±10.25	140.9 ± 16.97a	147.5 ± 28.28a,b
DBP (mmHg)	77.14±7.8	88.92± 14.14a	95.78 ± 21.21a,b

Statistical analysis was done by Anova (post hoc test : Bonferroni.[a]: Statistically significant from control group at p<0.05; [b]:Statistically significant from CAD WNDM at p<0.05.

The occurrence of CAD was computed in relation to age, BMI, and hypertension. Age has not been considered to be a modifiable risk factor but, it out ranks all other factors like lipids, and blood pressure,—as a predictor of clinical events. Conventional analyses do not distinguish between the biological changes of ageing within arteries—the non-modifiable effects of disintegration of tissues over time—and those produced by exposure over time to risk factors such as atherogenic dyslipoproteinaemia (8). The incidence of CAD was high among the patients of the age group 51-60 (77%) in CAD with type 2 DM patients when compared to CAD without DM (46%).

Table 2 Relation of Coronary Artery Disease to different variables

Variables		CAD WNDM N(%)	CAD WDM N(%)	P value
Age	40-50	15 (29 %)	1 (2%)	
	51-60	25 (48 %)	45(79%)	0.0001
(years)				
	61-70	12 (23%)	11(19 %)	

	18.5-24.9	1(2 %)	6(11 %)	
BMI	25. - 30	28(54 %)	12(21 %)	0.0004
(kg/m2)	>30			
		23(44 %)	39(68%)	

Statistical analysis was done by Chi square .Statistically significant from CAD WN DM at p<0.001.

TABLE 3.Severity of CAD among the study subjects.

CADsubjects		Severity of CAD		
	Grade I	Grade II		Grade III
CAD WNDM	24(46%)	15(29%)		13(25%)
CAD WDM	9(16%)	12(21%)		36(63%)

Statistical analysis was done by Chi square test. According to the percentage of stenosis and involvement of coronary vessels, severity of CAD was assessed and classified as Grade I (mild), Grade II(Moderate) and Grade III(Severe) (Table 3) . Analysis on frequency of distribution has revealed that severity was significantly high in CAD WDM. A significantly high percentage of multi vessel atherosclerosis was observed in CAD WDM when compared to CAD WNDM. These findings showed a delayed recognition of CAD in type 2 DM.

The typical symptoms of cardiac ischemia are often masked in diabetic patients. Hence the pathological events are not identified at the preliminary stages .This might be the reason for the observed high frequency of grade III severity in CAD WDM and high percentage of CAD related mortality in diabetic patients as observed in various epidemiological studies (9).

Table 4 .Biochemical Parameters of study subject

Parameters	CONTROL(N=71)	CAD WNDM (N=52)	CAD WDM (N=57)
FBS (mg/dL)	85.04±6.2	85.65 ± 5.657	147.05 ± 30.4a,b
PPBS (mg/dL)	101.81±5.1	109.4± 7.071	403 ± 53.7a,b
Insulin (µIU/ml)	8.41±0.334	8.76 ± 5.37	22.93 ± 3.25a,b
HOMA - IR	1.77±0.33	1.8±0.445	9.7 ± 1.1 a,b
Total cholesterol (mg/dL)	169±8.0	182.2 ±8.485a	197.49 ±7.77a,b
Serum Triglycerides(mg/d L)	I21.35±11.28	131.6 ±6.4	157.5 ±8.5a,b
HDL cholesterol(mg/dL)	41.48±5.59	38.4 ±2.07a	35.05 ±1.44 ab
LDL cholesterol(mg/dL)	104.48±33.5	129.50 ± 8.8 a	144.25 ±8.2a,b
VLDL (mg/dL)	24.27±2.26	26.3±13.01	31.5 ± 9.617a

Statistical analysis was done by Anova (post hoc test : Bonferroni) [a]:Statistically significant from control group at p<0.05;[b]: Statistically significant from CAD WNDM (p<0.05.).

There was significant (p <0.05) increase in mean .Fasting blood glucose in CAD WDM when compared to CAD WNDM and control. (Table 4). The mean Post Prandial blood glucose in CAD WDM was significantly high (p<0.05) when compared to CAD WNDM and control. The CAD WDM patients had significantly high insulin level (p<0.05) when compared to CAD WNDM and control. The CAD WDM patients had significantly high IR level (p<0.05) when compared to CAD WNDM and control. One of the basic factor which was found to be significantly elevated in CAD WDM group when compared to other two groups was Insulin resistance – which is the underlying defect in more than 90% of patients with T2DM.

The athero-thrombotic process that underlies cardiovascular diseases implies a central role for cholesterol metabolism. The level of total cholesterol was significantly high in CADWDM and CADWNDM) when compared to control. But no difference was observed between the CAD WDM and CAD WNDM. The triglyceride level in CAD WDM was significantly high (p<0.05) when compared to CAD WNDM. .Also the HDL level in CAD WDM was found to be low when compared to normal controls but was not significant in CAD WDM Vs

CAD WNDM. LDL level was significantly(p<0.05) high in CAD WDM when compared to CAD WNDM and control subjects.

In the present study cholesterol level was found to be significantly high in CAD patients. As its concentration increases, Cholesterol-rich apoB containing lipoproteins may infiltrate the subendothelial space and initiate an inflammatory process that leads to atherosclerotic lesions. This progresses to fibrous plaques, with necrotic scores. Abnormal concentration of cholesterol also increase platelet aggregation, which exacerbates the severity of athero-thrombotic process. Hence cholesterol metabolism occupies a central role in the pathophysiology of CVD and found to be high in CAD patients(10).

In the study, plasma triglyceride and VLDL were found to be significantly high in CAD WDM when compared to control and CAD WNDM. Hyper triglyceridemia has been found to be one of the most consistent finding in T2 DM . Evidence from both animal and human studies implied that insulin resistance(IR) which causes increased synthesis and/or decreased clearance of VLDL – has been an important underlying cause of hypertriglyceridemia in subjects with T 2DM (11).

One of the major abnormalities in IR was found to be hepatic overproduction of VLDL(19) . It influence hepatic VLDL production by increasing the free fatty acid flux to liver. It also reduce VLDL and IDL catabolism by reducing the activity of lipoprotein lipase and decrease the hepatic uptake of VLDL and IDL. These could have been the reason behind the significantly high level of TG and VLDL in CAD with diabetes subjects.

The present study has shown that LDL level was significantly elevated in CAD with diabetes subjects when compared to the CAD without diabetic group and normal controls. Reports have revealed an increased capacity for VLDL-TG secretion and an increased residence time of triglycerides in poor glycemic control condition of T2DM or in marked insulin deficiency. Insulin inhibits the production of large, buoyant VLDL particles and overproduction of these particles in patients with DM in the postprandial state **.** It has also been reported that clearance of LDL was limited due to the decreased availability of LDL receptors. So the high IR reported in CAD WDM might be the etiology behind the observed high level of LDL (12)HDL-C has been considered as an anti atherogenic lipid factor as it helps in reverse cholesterol transport. Furthermore HDL particles has been found to inhibit vascular inflammation and has antioxidant and antithrombotic properties. They enhance endothelial function, promote endothelial repair, increase angiogenesis, suppress the production and mobilization of monocytes and neutrophils from bone marrow (13).

In our study, HDL-C level has been found to be significantly lowered in CAD patients when compared to normal. These results draw a parallel with the existing reports (14). The reduced HDL cholesterol levels found inCAD WDM may be due to the high Apo E-containing triglyceride-rich lipoproteins found in these patients. Apo E is involved in HDL catabolism and can transfer from triglyceride-rich lipoproteins to HDL . Furthermore, when present on HDL particles, apo E is predominantly associated with LpAI/AII (Lipoprotein lipase AI/AII)particles. Therefore, the elevation of circulating apo E-containing triglyceride-rich lipoproteins could lead to increased transfer of apo E to LpAI/AII and enhanced catabolism of this fraction. (15).

Reports revealed that diabetes can double the risk of CVD even when the major risk factors are controlled(24)Since conventional coronary risk factors were not able to explain the increased incidence of CAD, novel risk factors were analyzed to understand its value in area of preventive cardiology .The amino acid homocysteine (Hcy) is a unique contender for the study of various pathological conditions, namely vascular diseases , due to its multiple roles in different pathways leading to atherosclerosis (16). The Hcy theory offers an explanation for observations on human arteriosclerosis that are difficult to explain by the conventional cholesterol/fat approach(17).There was significant (p <0.05) increase in CAD with diabetes as compared to control subjects. In the present study ,the hcy level in control group , CADWDM and CADWNDM was 7.56±1.891, 14.04 ± 1.202 and 17.9 ± 0.424 respectively. The elevated level of hcy was observed in CAD WDM ,when compared to the CADWNDM. Similar results were achieved by the studies of Drzewofki *et al* and they observed that patients with bad metabolic control of diabetes had significantly higher hcy level in comparison to diabetics with normal HbA1c levels. One of

the major reasons for this elevation may be deficiency of vitamin B6,B12 and folic acid, which are the cofactors of Hcy metabolism (18) .Earlier studies has shown ,lowered level of these –vitamin in diabetes patients which might be due to the increased excretion of vitamin B complex and decrease absorption of these vitamins in presence of metformin (19).

Table 5 :Distribution of subjects based on severity

Homocysteine (µmol/L)	CAD WITHOUT DM		
	GradeI	GradeII	GradeIII
>12 µmol/L	2%	23%	48%
<12µ mol/L	17%	6%	4%
	CAD WITH DM		
	GradeI	GradeII	GradeIII
>12 µmol/L	14%	19%	61%
<12µ mol/L	2%	2%	2%

Hcy :homocysteine .Statistical analysis was done by Chi square test.

Table 5 displays highly significant positive correlation between the hcy level and severity of CAD**.** In studies with a prospective design, the association of hyperhomocysteinemia (HHcy) with vascular disease is weaker; nevertheless, it is statistically significant in most cases .Studies have shown that an increase by 5 µmol/L of hcy can increase the CAD risk by as much as cholesterol increase of 20 mg/dl (20). Several studies have shown that sites of adverse effects of hcywere endothelial surface, vascular smooth muscle cells, connective tissue, interaction with plasma lipoprotein, clotting factors and platelets. Hcyhas been found to exist either in reduced (a thiol) or oxidized form.Oxidation of two hcy molecules resulted in the production of disulphide,two protons(H+) and two electrons.(e⁻).the presence of metal ions and oxygen causes the autooxidization ,resulting in the generation of superoxide anion radical and H_2O_2. Hence hcy has been found to exert its atherogenic effect by promoting oxidative damage in endothelial cell with generation of superoxide anion radical and H_2O_2.This may promote oxidation of LDL and deactivation of NO - the major endothelial derived vasodialator. Decreased bioavailability of NO may lead to vasoconstriction ,platelet aggregation and monocyte adhesion –all of which promote atherogenesis and pathological cardiovascular events (21).These observations and report highlights the fact that hcy can be considered as a precious non traditional risk factor for CAD.

In CAD with diabetes population BMI showed significant positive association with Hcy (r=.413, p<0.001).In our study a significant negative correlation was observed between hcy and HDL and a positive correlation between hcy and LDL.(Table).Similar results were observed in the studies of Rossa Maria *et al* in elderly population and in the Frahmingam and Hoorn;s study. Studies using cystathionine synthase knock out mice also has demonstrated a negative correlation. Studies in these mice has shown that HHcy was associated with decreased activity of hepatic thiolase and serum LCAT. Evidences on these studies suggested that hcy reduces the liver expression of apoA1 and there by reduce the level of apo A1 and HDL-C. Liao et al has found that HHcy has an effect at the post translational level of apoA1 expression .In addition ,studies have found that hcy reduce the rate of plasma cholesterol esterification by LCAT and increased clearance of HDL-CE from the plasma. Another study has shown that hcy decrease the apo A1 expression by lowering the transcription factor PPAR(Peroxisome proliferator-activated receptor that participate in the apo A1 transcription. PPAR is also involved in the increased expression of cholesterol 7 hydroxylase , a rate limiting enzyme involved in bile acid synthesis. Therefore a significant increase of hcy level in the present work could be responsible for the pathogenesis of C AD (22).Quejeq et al has observed a positive correlation between total hcy level and LDL level, in patients with MI. It is well known that 75-80% of total plasma hcy circulates bound to plasma proteins mainly by means of disulphide bonds with thiolic residues of circulating proteins (23).It has been shown that significant amount of hcy (25-30%) gets bound to lipoprotein especially LDL and VLDL which were found to have high binding capacity for hcy. .ApoB are particularly rich in cysteine residues and provide optimal binding sites for hcy molecule. Hence higher Apo B content may explain ,the higher hcy binding capacity of LDL with respect to VLDL and other lipoproteins .

CONCLUSION
To stumble on and comprehend the role of non-conventional biomarker Hcy in the diagnosis of CAD, the following study was undertaken. As Diabetes mellitus forms a major non-communicable disease carrying the greatest risk for CAD, the present study have shown that the levels of serum hcy was high in CAD with T2DM. Severity of CAD was found to be positively correlated to the level of hcy. Hence Hcy plays a role in atherosclerosis progression, explaining its role as a novel independent marker .

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