

EVALUATION OF SERUM HOMOCYSTEINE IN PATIENTS WITH UNSTABLE ANGINA A NEW POTENTIAL RISK FACTOR

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ABSTRACT

Atherosclerotic coronary artery disease causing myocardial ischemia may manifest itself either as acute myocardial infarction (AMI), unstable angina, effort angina or sudden death. A number of risk factors are known to predispose to coronary artery disease. Some of these such as age, sex and family history can not be modified, whereas others can be changed, but these classical risk factors cannot fully explain why some people develop myocardial infarction and stroke while others do not. Additional factors may have a role in the pathogenesis of atherosclerosis. We have evaluated the level of a new risk factor homocysteine in patients suffering from unstable angina of our local population. The estimation was done by FPIA method on IMX and we found significantly high levels of total homocysteine in patients suffering from unstable angina.

INTRODUCTION

Acute myocardial infarction and unstable angina are the leading causes of death in all racial and ethnic groups (Rashid and Butt 2000). In Pakistan 47% of all cardiac deaths are due to coronary heart diseases (Samad 1995). Hospital based data from Pakistan as far back in 1972 showed a tremendous increase in the prevalence of coronary artery disease (Rasheed and Samad 1999). Coronary artery disease is a condition of diverse etiology but a disturbance between oxygen supply and demand is common to all. The most common cause of ischemia is atherosclerotic disease of epicardial coronary arteries (Ahmed and Ahmed 1995). It often strikes at the height of one's working career. About 37 % of American males and 29 % American Females who die of CAD are younger than 55 years (Farmer and Gotto 1997).

The classical modifiable risk factors for atherosclerosis are High cholesterol, blood pressure and Smoking while non-modifiable

factors are age, sex and family history. These classical risk factors however cannot fully explain why some people develop myocardial infarction and stroke while others do not. Additional factors may have a role in the pathogenesis of atherosclerosis, so new preventive and diagnostic strategies may have to be used. Elevated plasma homocysteine have recently been identified as a potential risk factor for atherosclerosis and coronary heart disease (Malinow *et al.*, 1999). In our study we have evaluated this new emerging potential risk factor for atherosclerotic coronary artery disease.

Homocysteine is a curious sulphur-containing amino acid formed during methionine metabolism. It can dimerise to homocystine, or form disulphide bonds with proteins to form so-called 'protein-bound' homocysteine. In plasma about 80 % of homocysteine is protein bound (Kilmer McCully 1998).

The measurements of total plasma or serum homocysteine represent the sum of

oxidized (as homocysteine or the homocysteine-cysteine mixed disulfide) and protein-bound homocysteine. Homocysteine contains a reactive sulfhydryl group that can react with plasma constituents, and this may promote oxidative damage and induce the oxidation of low-density lipoprotein (LDL). An elevated homocysteine level therefore induces thrombogenicity, causes pro-coagulant state and promotes the proliferation of smooth muscle cells.

SUBJECT AND METHOD

The proposed study was carried out in the department of Biochemistry, Basic Medical Science Institute, Jinnah Postgraduate medical Centre Karachi in Collaboration with National Institute of Cardiovascular Diseases Karachi. Subjects were divided into two groups, Group A and Group B.

Group A: Twenty-one subjects with no history of IHD served as controls. They were age and sex matched with subjects in-group B.

Group B: Twenty-one subjects were placed in this group. These were the Patients having unstable angina.

Controls were free of overt disease and there was no clinical or ECG evidence of ischemic heart disease in any of these individuals.

The data collection for various groups was standardized through the use of similar methodology, protocol and procedure using a standard questionnaire. The questionnaire provided information about type of work, smoking habit, medical history of cardiovascular disease, hypertension and family history of coronary heart disease. Smoking habits of subject was grouped into current smokers and non smokers, weight was measured on a balance scale while participants were without shoes and heavy outer garments, height was measured in the

standing position following weight measurement. Blood pressure of subject was measured twice in the right arm after 5 minute of rest, using a standard mercury sphygmomanometer. Values from the second measurement were used in this study. Diagnosed unstable angina cases were selected from National Institute of cardiovascular disease Karachi. The subjects were asked to fast for 10-12 hours and 6 ml of blood was collected from the antecubital vein, (after all aseptic measures) while the subjects were sitting up right. Sampling was done between 0700 and 0900 hours.

Specimen Handling and Storage

Strictly predefined protocol was used for specimen preparation. Blood was collected in a gel Barrier silicone coated neotube from Nipro Japan. The additive free blood-tubes were put on ice in the icebox immediately after collection. Whole blood tubes were kept at room temperature until clotting was complete. Those samples that showed sign of haemolysis were discarded. Samples were centrifuged at 1000 rpm for 10 minutes within one hour of collection, serum was separated and stored in aliquots in deep freezer at minus 20°C until assayed (within one month). Samples were analyzed in one run at the end of the study, to omit between run analytical variations except serum glucose that was analyzed within four hours of sample collection. Serum total cholesterol, HDL-C, triglycerides and glucose were analyzed enzymatically, using the kits supplied by SPINREACT, Spain. LDL-C was calculated by the Friedwald formula. Serum total homocysteine was analyzed by FPIA (Fluorescence Polarization Immunoassay) using the IMX analyzer of Abbot Laboratories Pakistan for which we are extremely thankful to their diagnostic division.

RESULTS

A total of forty-two subjects were studied. Table 1 shows the mean values and

comparison of age, weight, height, body mass index and smoker status, of control (group A) and case (group B) subjects. Twenty-one healthy normal volunteers, nineteen (19) males and two (2) females and Twenty-one patients, fifteen males (74.4%) and six females (28.6 %) having unstable angina were placed in group A and B. When age, weight, height and BMI of control subjects were compared with group B patients, no significant difference of age and weight was observed, while height in-group B was significantly low ($p < 0.001$) and body mass index was high ($p < 0.05$) in-group B as compared to control. In relation to smoking habit, none of the subjects was smoker in control group. In-group B 42.9% (9) patients were smokers, which is significantly high ($p < 0.001$) as compared to control group. Rest of the variables in table 1 are non-significant as compared to control group. Table 2 shows mean values of hypertension, systolic blood pressure diastolic blood pressure, heart rate and family history of ischemic heart disease of control and group B, when history of hypertension was compared between control and group B, control subjects were non hypertensive. In-group B, 28.6 % (6) patients were hypertensive and the difference is significantly high ($p < 0.05$) as compared to control. When systolic blood pressure (SBP), diastolic blood pressure (DBP), family history of ischemic heart disease (F/H of IHD) and Heart rate (H/R) were compared among control and group B, non-significant changes were observed. Table 3 shows the mean values of serum glucose, triacyl glycerol, total cholesterol of control and group B subjects. The value of serum glucose, Triacylglycerol and Total cholesterol of group B were non-significant when they were compared to control. Table 4 shows comparison of the mean values of serum. HDL-C, LDL-C, VLDL-C and total Homocysteine, of group B patients with control. The mean value of high-density lipoprotein cholesterol of group B was significantly low ($p < 0.001$) as compared to control subjects. The values of low-density lipoprotein cholesterol of group B, was

significantly high ($p < 0.001$) as compared to control subjects. VLDL cholesterol was non-significant in-group B as compared to control. Serum total homocysteine of group B was significantly high ($p < 0.001$) as compared to control subjects.

DISCUSSION

Ischemic heart disease (IHD), also designated as coronary heart disease (CHD), refers to a group of closely related syndromes caused by an imbalance between the myocardial oxygen demand and blood supply. The most common cause of this disease is reduction in coronary arterial blood supply due to atherosclerosis of the coronary arteries. (Eikelboom *et al.*, 1999). Akosh *et al.* (2000), have reported that history of smoking was independently associated with premature coronary heart disease and they found a higher percentage of smokers with ischemic heart disease than control. Gensini *et al.* (1998), show that smoking is responsible for 29% of total death from coronary heart disease. In our study also the percentage of smokers is significantly high ($P < 0.001$) in patients with unstable angina as compared to control subjects.

Elevated blood pressure (Hypertension) is a significant strong and independent risk factor for coronary artery disease, both in men and women (Brochier *et al.* 1998). We have found a significantly high ($P < 0.001$) percentage of hypertension in patients with unstable angina disease as compared to control subjects. The study of Hamstern and Fair (1987) shows the presence of familial predisposition to coronary artery disease (CAD) in a substantial number of men with myocardial infarction occurring at a younger age. Whether a family history, contributes to development of coronary artery disease independent of other risk factors is unknown. However, In our study we could not find any significant difference of family history of IHD between controls and patients with unstable angina. The dyslipidemia, most clearly associated with increased risk for

Table 1
Comparison of age, weight, height, BMI and smokers status of group A (control)
and group B (patients with unstable angina) subjects

The values are expressed as mean $\pm$ SEM.
The number of observation and units are given in parentheses.

Groups Male/female	Age (years)	Weight (Kg)	Height (m)	BMI (Kg/m ²)	Smokers
Group-A (Control) 19/2 (21)	46.38 ± 1.38	71.0 ± 1.17	1.71 ± 0.02	24.53 ± 0.64	0
Group-B (Patients) 15/6 (21)	44.86 ± 1.15	63.29 ± 3.23	1.60 ± 0.03	25.62 ± 0.96	9 *** (42.9 %)

BMI: Body mass index

***P<0.001 significant as compared to control group

Table 2
Comparison of hypertension, systolic blood pressure, diastolic blood pressure, heart rate
among group-A (control) and group-B (patients with unstable angina) subjects.

The values are expressed as mean $\pm$ SEM the number of observation
and units are given in parentheses.

Groups	Hyper-tension %	SBP (mm/Hg)	DPB (mm/Hg)	Heart Rate (b/min)	FH of IHD %
Group-A (Control) 19/2 (21)	0	127.14 ± 1.71	80.48 ± 0.76	78.29 ± 0.97	2 (9.5 %)
Group-B (Patients) 15/6 (21)	* 6 (28.6)	126.19 ± 3.35	77.62 ± 2.92	74.48 ± 2.25	5 (23.8 %)

*P<0.05, significant as compared to control

SBP : Systolic Blood Pressure

DBP : Diastolic Blood Pressure

FH of IHD : Family History of Ischemic Heart Disease

CAD is hypercholesterolemia, particularly elevated plasma levels of cholesterol carried in LDL. The association between elevated blood cholesterol and CAD has been established in observational and interventional epidemiological studies.

In our study we report no significant difference between the total cholesterol and triglyceride levels of case (patients with IHD) and control subjects. Although the mean level was slightly higher in the cases, but

statistically it was non-significant. Similarly we report lower mean levels of HDL-C, with a significant difference (P<0.001) in-group B subjects as compared to control. We have also observed that, the patients with unstable angina have significantly (P<0.01) high levels of LDL-C as compared to control. Clarke et al. (1998), and Robinson et al. (1998), reported in their studies that increased levels of plasma homocysteine are common in patients with stroke, coronary artery disease and peripheral vascular

Table 3

Comparison of fasting serum glucose, Triacylglycerol, total cholesterol among group-A (Control) and Group-B (patients with unstable angina) subjects

The values are expressed as mean $\pm$ SEM. The number of observation and units are given in parentheses.

Group	Serum glucose (mg/dl)	Triacylglycerol (mg/dl)	Total Cholesterol (mg/dl)
Group-A Control (21)	84.38 ± 0.23	140.52 ± 9.01	166.14 ± 11.89
Group-B Patients (21)	74.57 ± 0.31	146.09 ± 11.92	191.33 ± 8.35

Table 4

Comparison of fasting high density lipoprotein cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and total Homocysteine among Group-A (control) and Group-B (Patients with Unstable angina) subjects

The values are expressed as mean $\pm$ SEM the number of observation and units are given in parentheses.

Group	HDL-C (mg/dl)	LDL-C (mg/dl)	Homocysteine (μ mol/l)
Group-A Control (21)	44.95 ± 1.75	93.08 ± 11.43	10.57 ± 0.31
Group-B Patients (21)	*** 34.0 ± 1.90	** 128.11 ± 7.51	*** 16.84 ± 0.93

P<0.01, *P<0.001 significant as compared to control group

HDL-C High density lipoprotein cholesterol

LDL-C Low-density lipoprotein cholesterol

VLDL-C Very low density lipoprotein cholesterol

disease, and that it confers an independent risk of atherosclerosis. In 1995, meta analysis of 27 observation studies involving a total of approximately 4000 participants, it was reported that hyperhomocysteinemia, defined as plasma homocysteine level above the 90th or 95th percentile of level in control, was associated with an increased risk of fatal and non fatal atherosclerotic vascular disease in the coronary and cerebral circulation. We have found a significantly (P<0.001) higher values of total homocysteine in patients with unstable angina as compared to control

subjects which is in agreement with the above mentioned analysis.

In conclusion, we have observed that hypertension, smoking, deranged lipid levels i.e. increased LDL cholesterol, increased VLDL cholesterol, and decrease HDL cholesterol, as well as a raised serum total homocysteine levels are strongly associated with ischemic heart disease. Regardless of all other risk factors, serum homocysteine appears to be a strong marker of ischemic heart disease.

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