COMPARISON OF SERUM LIPIDS, LIPOPROTEINS AND APOLIPOPROTEINS IN PATIENTS UNDERGOING CABG WITH HEALTHY ADULTS

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OBJECTIVE:

To assess the pattern of plasma lipoproteins in CAD patients including comparison between diabetic and non-diabetic subgroups.

METHODS:

This case control study was conducted at National Institute of Cardiovascular Diseases (NICVD) Karachi on patients admitted for CABG from June, 2000 to June, 2001. A total of 100 patients and 100 healthy subjects were enrolled during the study. 38 patients (38%) were diabetic with mean age of 46.16 years and 62 patients (62%) were non-diabetic with mean age of 45.26 years. Serum lipids, apolipoproteins A, apolipoproteins B and Lp(a) of each subject were analysed. One way ANOVA and 95% confidence interval for mean were applied to find the association of CAD with age, BMI, glucose, cholesterol, triacylglycerols, HDL-c, LDL-c, and Lp(a).

RESULT:

CABG patients had significantly higher blood TAG levels than controls. Similar results were observed for HDL-c, which was significantly lower when compared with control subjects. Apo-B and Lp(a) in controls were significantly lower than both diabetic and non-diabetic CAD patients. The level of Apo-B in diabetic CAD was significantly higher when compared with control subjects.

KEY WORD:

Lipoprotein (Lp.), Apolipoprotein (Apo), Triacylglycerols (TAG), Coronary Artery Bypass Graft (CABG), Coronary Artery Disease (CAD)

INTRODUCTION

Atherosclerosis is a disease process that involves disturbance in Lipid metabolism, blood coagulation and chronic inflammation of vessels in association with underlying genetic and environmental factors[1].

Atherosclerosis is the principal cause of death in Western Civilization[2]. As a result of complex interaction between different factors, disturbed flow, vessel wall abnormalities including inflammation with increased endothelial permeability, endothelial activation and monocyte recruitment, smooth muscle proliferation and migration with matrix synthesis are observed during the process of Atherosclerosis. Degeneration with lipid accumulation, as well as possible cytotoxic effect of oxidized lipids are also major processes in the pathogenesis.

It is well recognized that atherosclerosis is a multi-factorial process that begins in childhood and has clinical and biochemical manifestations in middle to late adulthood. Available data suggests that a large size of population is effected by this condition. Therefore, the identification of subjects at higher risk of developing atherosclerosis is an important public health issue. Epidemiological and biochemical studies demonstrate that atherosclerosis is a complex process in which both environmental and genetic

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factors promote atheroma formation. Risk factors such as male gender, increasing age, hypertension, diabetes and a family history of premature coronary artery disease, obesity, physical inactivity, cigarette smoking, elevated plasma low density lipoprotein cholesterol (LDL-c) and decreased high density lipoprotein cholesterol(HDL-c) have been implicated as independent predictors for coronary artery disease[4] in both prospective[5], case control[6,7] and epidemiological studies. Cholesterol, as normal metabolite, is usually not harmful unless it accumulates in excessive quantity due to excessive production or intake. An interruption of its use in metabolic pathways such as in famillal hyper - cholesterolemia can lead to certain problems. Most cholesterol in the plasma of fasting normal subjects is carried by LDL-c, much smaller proportion is found in VLDL-c and HDL-c. In contrast, most endogenous TAG is carried by VLDL-c. Studies have reported that elevated plasma concentration of LDL-c is associated with accelerated atherogenesis[5,8,9]. The major apolipoproteins of LDL-c and HDL-c particles, namely apolipoprotein B and apolipoprotein A respectively, are strongly associated with the presence of coronary artery disease. An increased levels of apolipoprotein B and low levels of apolipoprotein A have been associated with increased risk for atherosclerosis[10,11].

Apolipoproteins (Apo) play very important role in the synthesis and catabolism of plasma lipoproteins. These are also involved in lipid transport and as activator of certain enzymes associated with lipid and lipoprotein metabolism resulting in various metabolic states and diseases. Apolipoproteins are proteins component of plasma lipoproteins which consist of a core of TAG and cholesterol esters and a peripheral region of phospholipids, sphingolipid and protein. They are designated ApoA-I, ApoA-II, ApoB-48, ApoB-100, ApoC-1, ApoC-II, ApoC-III, Apo-D, ApoE-I, ApoE-II, ApoE-III, ApoE-IV, ApoF, ApoG and ApoH[12].

They also play very important role in the synthesis and catabolism of lipoproteins and as a cofactor in activation or inhibition of certain enzymes, viz Lecithin Cholesterol Acyl Transferase, (LCAT), hepatic lipase, lipoprotein lipase. These are associated with lipid and lipoprotein abnormalities either due to an excess or deficiency of a apolipoprotein leading to a specific disease or a syndrome[13]. ApoA-I is a major protein component of high density lipoprotein (HDL-c) and a minor component of chylomicrons and very low density lipoproteins (VLDL). ApoA-II is the second most abundant protein in HDL-c comprising approximately 20% while ApoA-1 constitutes about 70% of its contents[14]. High levels of ApoA-1, ApoA-II or both are predictors of decreased risk of coronary artery disease. The role of HDL-c and its main protein component, apolipoprotein A-1, is recognized as antiatherogenic by reversing cholesterol transport and anti-inflammatory mechanisms. Diabetes mellitus, hyperlipidemia, hypertension, obesity and insulin resistance are tightly interrelated. Each one is an independent risk factor for atherosclerotic events. The pathogenic mechanisms involved are still unresolved and highly controversial. According to National Diabetic Data Group[15], the levels of lipoproteins such as LDL choiesterol may not be abnormal in patients with diabetes mellitus; however Lipoproteins may be glycated resulting in abnormal function. Low density lipoprotein cholesterol containing glycated ApoB-100 interact with platelets and vascular endothelium to increase thromboxane production and decrease thrombolytic prostaglandins. This favours thrombosis in micro and macro circulation in patients with diabetes mellitus. Plasminogen activator inhibitor is also increased in diabetes. Glycation of lower HDL-c level, characteristic of type II diabetes, causes further impairment of reverse cholesterol transport resulting in accelerated macrophage uptake of LDL through scavenger receptor pathway. A number of epidemiological and clinical studies have implicated hyperinsulinemia and insulin resistance in increased frequency of CAD[16,17].

METHODS:

Patients admitted in NICVD for coronary artery bypass graft (from June 2000 to June 2001) were the main source of cases included in this study. One hundred patients, undergoing CABG surgery, were selected after carefully applying the inclusion and exclusion criteria. Equal number of matched control group was included in this study. Informed consent of each subject was taken. History from patients and controls was taken on a questionnaire designed for this study. Detailed history of present and past illness,

socioeconomic status, family history of ischemic heart disease (IHD), and other coronary risk factors were recorded. Current medications were reviewed and particular attentation was paid to lipid lowering drugs.

The study was open to comparatively young men and women between 25-55 years of age with documented coronary artery diseases (CAD). Recording of the coronary risk factors namely cigarette smoking, hypertension, family history of CAD, diabetes mellitus (DM), obesity, sedentary life was ensured. Patients on any lipid lowering agents, endocrine disorders like hypothyroidism, history of malignancy, hepatic disease and malabsorption were excluded from the study. WHO criteria for diagnosis of diabetes mellitus was followed. A person was considered diabetic if he or she was already on anti-diabetic medication irrespective of current blood glucose level. A person was also considered diabetic with fasting venous plasma glucose of more than 126 mg/dl obtained on two separate occasions or venous plasma glucose level more than 200 mg/dl after 2 hours of 75 gm glucose ingestion on two separate occasions. Venous blood sample (5-10 ml) was obtained from each patient in the morning after 12-14 hours overnight fast from 100 randomly selected Pakistani adults admitted in surgical ward for CABG at NICVD Karachi. Control samples were collected from healthy adult volunteers from general population following the same procedure. In control group, anyone with history of hypertension, diabetes mellitus, cardiovascular, neurovascular disease and primary of secondary dyslipidemia was excluded. Fresh samples were used for the estimation of blood glucose, total cholesterol, TAG, HDL-c. For long term storage, all serum samples were stored at -40oC. Total cholesterol and triglycerides levels were measured by conventional enzymatic methods (18). Apo-B and ApoA-I and Lp(a) were measured in fresh blood samples by immunoturbid metric technique (19). Analytical procedures were compliant with World Health Organization/ International Federation of Clinical Chemistry apolipoprotein standard program. All methods were fully automated (20,21), LDL-c cholesterol was calculated using statistical Fried Wald formula (22) and performed in AKU Research Laboratory Karachi. Statistical Package for Social Sciences (SPSS-version 10 for windows) program was used to perform statistical analysis. Age (in years), sex, glucose, total cholesterol, Triglycerides (TG), HDL-c, LDL-c, ApoA-I, ApoB, Lp(a) and diabetes mellitus were taken as predictor variables. Univariate analysis was performed primarily to see association with predictor variables. This included standard comparison of group means i.e. Student t-test, analysis of variance (ANOVA), logistic regression and calculation of odd ratio with 95% confidence interval to measure the strength of association.

RESULTS:

A total of 100 control subjects and 100 patients suffering from coronary artery disease (CAD), documented on coronary angiography, were investigated during present study. The distribution of both cases and controls, on the basis of age, sex and body mass index (BMI) are given in table I & II. 76% patients were male and 24% were female. Whereas in control subjects, 78% were male and 22% were female. The mean age of male patients was 45.88+4.44 years and female patients 44.71+5.49 years. Majority of our patients was in relatively younger age group. The mean age of male and female control subjects were 44.43+4.68 years and 45.59+4.91 years respectively. There was no statistically significant difference between male and female in both cases and control groups (Table-I). Based on BMI, the subjects were categorized according to the criteria set by National Heart Lung Blood Institute (NHLBI) (23). In cases, 6% patients were under-weight, 43% in desirable range, 41% over-weight, and 10% were obese. In control subjects, 4% were underweight, 45% in desirable range, 45% overweight and 6% were obese. The BMI of cases was not statistically different from that of controls (Table-II).

The lipid profile of cases and controls is shown in Table - III. The mean total cholesterol level of patients was 188.75 mg/dl and in control 183.72 mg/dl, the difference was not statistically significant (95% CI - 7.27, 17.33). However, the mean levels of HDL-c among patients and control group were 37.97 mg/dl and 42.27 mg/dl respectively. This difference was statistically significant (95% CI-6.11, -2.49). There was no significant difference in the mean LDL-c between cases and controls (119.42+51.07 vs 109+29.92). Significantly increased level of TAG

was found in patients (191.03+93.32 mg/dl) compared to control subjects (157.11+83.22 mg/dl), with 95% confidence interval of 9.22 to 58.62.

The ratio of LDL-c to HDL-c (Table - III) was significantly higher in cases (mean 3.44) compared to control (mean 2.66) (95% CI - 1.39 - 0.17). In contrast, the ratio of total cholesterol to HDL-c was not statically significant between the two groups. Table-IV shows a summarized distribution of apolipoproteins between cases and control subjects. Apo-A was significantly lower in cases (mean 123.60 mg/dl) than controls (mean 132.40 mg/dl) (95% CI- 17.20, - 0.40). The mean levels of Apo-B was 157.50 mg/dl in cases and 104.90 mg/dl in controls. This difference was highly significant. Mean Lp(a) was 39.04 mg/dl in cases and 26.30 mg/dl in controls (95% CI 5.80, 19.70). This difference was also highly significant. In our patient population, 38 subjects were diabetic and the remaining 62 were non-diabetics. We compared glucose and lipids profile in controls with diabetic and non-diabetic patients and results are presented in Table-V. As expected, glucose level in control and non-diabetic groups was not

TABLE – I (AGE & SEX DISTRIBUTION)

Variable	Male (Mean±SD)	Females (Mean±SD)	Total (Mean±SD)
Control	44.43±4.68 (n=78)	45.59±4.91 (n=22)	44.30±4.67 (n=100)
Patient	45.88±4.44 (n=76)	44.71±5.49 (n=24)	45.60±4.71 (n=100)

TABLE – II (BMI DISTRIBUTION)

BMI (KG/M^2)	CASES	CONTROLS
Underweight (20 or less)	6.00%	4.00%
Desirable (20-25)	43.00%	45.00%
Overweight (25-30)	41.00%	45.00%
Obesity (>30)	10.00%	6.00%

TABLE – III (LIPID PROFILE DISTRIBUTION)

variables	Case(n=100) Mean ±SEM	Control n=100) Mean ±SEM	95% CI for difference of mean
Total cholesterol (mg/dl)	188.75±52.50	183.72±33.55	(-7.27, 17.33) NS
Triacylglycerol (mg/dl)	191.03±93.32	157.11±83.32	(9.22, 58.62)**
HDL-C (mg/dl)	37.97±6.42	42.27±6.59	(-6.11, -2.49)***
LDL-C (mg/dl)	119.42±51.07	109.96±29.92	(-2.26, 21.17) NS
LDL-C / HDL-C ratio	3.44±2.97	2.66±0.83	(-1.39, -0.17)*
Total cholesterol / HDL-C Ratio	5.40±3.88	4.45±0.96	(-0.97, 0.017) NS

CI = Confidence Interval
* significant at <0.05 level.
** significant at <0.01 level.
*** significant at <0.001 level.
NS= Non significant

TABLE – IV (APOLIPOPROTEIN-A, APOLIPOPROTEIN-B & LP(a))

Variables	Case(n=100) Mean ±SEM	Control n=100) Mean ±SEM	95% CI for difference of mean
Apo-A (mg/dl)	123.60±30.50	132.40±29.70	(-17.20, -0.40)*
Apo-B (mg/dl)	157.50±84.17	104.90±30.92	(34.85, 70.35)***
LP(a) (mg/dl)	39.04±25.53	26.30±24.40	(5.80, 19.70)**

CI = Confidence Interval
* significant at 0.05 level.
*** significant at 0.001 level.

significantly different; whereas diabetic patients had significantly higher glucose level than both control and non-diabetic patients (P<0.05). Both diabetic and non-diabetic patients had significantly higher blood TAG level than control, but the difference between diabetic and non-diabetic patients was not significant. Similar results were observed for HDL-c, which was significantly higher in control group than both diabetic and non-diabetic groups.

The ratio of LDL-c/HDL-c in non-diabetics was significantly higher than controls but it was not different from diabetic patients; the difference between controls and diabetics was also significant. No statistically significant difference was observed for total cholesterol/ HDL-c ratio among control, diabetic and non-diabetic CAD patients. Apolipoproteins and Lp(a) comparison was done among controls, diabetics, and non-diabetics. ApoA was significantly higher in control group compared with cases. However, the difference between diabetic and non-diabetic CAD patients was not significant. ApoB and Lp(a) in control group was significantly lower than in both diabetic and non-diabetic patients. The difference in levels of ApoB in diabetics vs control group was highly significant.

TABLE – V DISTRIBUTION OF GLUCOSE, LIPID PROFILE, LDL-C, HDL-C, TOTAL CHOLESTEROL AND HDL-C RATIO, APO-A, APO-B, AND LP(a)

VALUES ARE EXPRESSED IN MG/DL AS MEAN

Variables	Control (n=100) 95% CI for mean	Non Diabetic with CABG (n=62) 95% CI for mean	Diabetic with CABG (n=38) 95% CI for mean
Age (Years)	44.43 (43.6, 46.36)	45.26 (44.07, 46.46)	46.16 (44.59, 47.72)
BMI (Kg/m^2)	24.94 (24.23, 25.65)	24.97 (24.16, 25.80)	26.60 (24.40, 26.78)
Glucose (mg/dl)	93.30^a(91.65, 94.95)	94.82^a(92.47, 97.17)	183.34^b(177.26, 189.43)
Cholesterol (mg/dl)	183.72^{ab}(177.06, 190.40)	197.42^a(184.40, 210.45)	174.61^b(157.52, 191.70)
Triacylglycerol (mg/dl)	157.11^b(140.54, 173.70)	189.19^a(166.0, 212.26)	194.13^a(161.92, 226.36)
HDL-C (mg/dl)	42.27^a(40.96, 43.68)	38.21^b(36.24, 40.18)	36.68^b(34.64, 38.72)
LDL-C (mg/dl)	109.96 (103.96, 115.96)	121.84 (109.04, 134.64)	116.46 (98.17, 132.76)
Apo-A (mg/dl)	132.40 (126.60, 138.28)	124.10 (116.64, 132.54)	122.76 (114.32, 132.20)
Apo-B (mg/dl)	104.86^b(98.72, 110.0)	148.91^a(139.10, 159.84)	171.42^a(130.50, 212.34)
LP(a) (mg/dl)	26.30^b(21.46, 31.14)	40.3^a(33.28, 47.43)	36.98^{ab}(30.08, 43.88)

Values denoted by different superscript letters indicate significant difference at P<0.05
CABG = Coronary artery bypass graft
CI = Confidence Interval

TABLE – VI UNIVARIATE ANALYSIS

Factors	Case	Control	OR	95% CI for difference of mean
Age group (Years)				
<40	15(15%)	22(22%)	1	
>40	22(85%)	85(78%)	1.598	(0.77, 3.30)
Sedentary Life				
Yes	28%	4%	12.57	
No	72%	97%	1	(3.68, 42.98)
Triacylglycerols (mg/dl)				
Normal	65%	82%	1	
Abnormal	35%	18%	2.453	(1.274, 4.723)
HDL-C (mg/dl)				
Normal	62%	89%	1	
Abnormal	38%	11%	4.96	(2.363, 10.42)
Apo-B (mg/dl)				
Normal	58%	72%	1	
Abnormal	42%	28%	1.862	(1.023, 3.360)
LP(a) (mg/dl)				
Normal	52%	70%	1	
Abnormal	48%	30%	2.164	(1.205, 3.848)

OR = Odd Ratio
CI = Confidence Interval

Table VI shows analysis of variance and calculation of odd ratio to measure the strength of association between CAD and some risk factors. Odds of having CAD were greater with sedentary life, lower HDL-c level, higher level of TAG, Lp(a), AopB and older age.

DISCUSSION:

Coronary artery disease (CAD) is multi-factorial in origin, so it is important to estimate the risk of developing or having coronary artery disease. Certain biochemical and clinical characteristics are useful in identifying relevant factors. These include age, sex, smoking, hypertension, diabetes mellitus (DM), obesity, high serum cholesterol, triacylglycerols, low HDL-c, high LDL-c, ApoA-1, ApoB, Lp(a), family history of CAD and physical inactivity. The present study was designed to sample CAD patients (both diabetic and non-diabetic) with normal control to analyze lipids, lipoproteins, apolipoproteins and Lp(a) of CAD patients of relatively younger age. The observations and results of this study were evaluated in light of previous studies available in literature.

Unfortunately the incidence of CAD in Pakistani population, particularly among younger age group, is reported to be higher than Western population of the same age group. The reason for this marked difference is still unknown. There is a derth of information regarding the status of aforementioned biochemical risk factors, such as specific lipoproteins, apolipoproteins and Lp(a).

In present study smoking was found to be present in 24%; diabetes mellitus in 38%; hypertension in 32%; family history of CAD in 31%; sedentary life in 28%; overweight in 39% and obesity in 7% of total cases. Our investigation revealed that 43% patients had three or more risk factors and only 13% had one risk factor, while 7% patients had none of the aforementioned risk factors. In evaluating the variables which influence the risk of CAD at younger age group, the results of present study are similar to most studies in literature describing positive relationship between CAD and smoking, diabetes mellitus, hypertension.

However, the most prevalent risk factors in our study are diabetes mellitus, overweight, hypertension and family history of CAD. A study conducted in 1992, called the four Cities (24), demonstrated the high prevalence of hypertension, dyslipidemia and smoking in Pakistani population which was comparable to Unites States Population (25). Considering higher number of relatively young individuals undergoing CABG, we thought it would be appropriate to identify risk factors that may be responsible for occurrence of this pattern of disease.

Although the occurrence of CAD in younger age group has been reported elsewhere (26,27) and a number of studies conducted in West indicated an early onset of CAD between 3%-6% (27,28). However, the incidence of CAD in younger age Pakistani population (15%) appear to be on higher side. In present study the number of CAD patients below 40 years of age were 15% which is four to five times higher than that in Western population. The aetiopathogenises of CAD in younger age group is not well established. A number of authors have investigated the frequency of risk factors in younger coronary artery disease patients with conflicting results. In study conducted by Weiburger et al in 1987 (29), smoking was present in 56% cases, positive family history of CAD in 14%, hypertension, diabetes mellitus and obesity were present in 3.33% each and increase serum lipids in 14% cases. In 1986, Kani et al (30) studied young Indian patients under 40 years (n =104) and found 76.2% smokers, 30.3% with raised serum cholesterol levels, 32.5%, hypertensive, 28.7% with positive family history of CAD and 5% diabetics.

Our study is comparable with most of the above studies regarding frequency of coronary risk factors. The most prevalent risk factors in our study were DM (38%) hypertension (32%). Univariate analysis was done to assess the association of CAD with the presence of abnormal levels of lipids, apolipoproteins, Lp(a), age and sedentary life style. In present study the variables independently associated with CAD are age, sedentary life, TAG, HDL-c, Apo-B and Lp(a). TAG was highly significant in age group older than 40 years; in younger patients (Age <40 years), Apo-B, Lp(a) and LDL-c were found to be more significant. Similar findings were reported by Jucques et al (31) in their case control study, Schaefer et al, Genest et al (32), Rosengeren et al (33). The Physiological role of Lp(a)

may interfere with intravascular thrombolysis and inhibit the streptokinase mediated breakdown of blood clots that triggers heart attacks. Apo (a) is highly homologous to PLASMINOGEN - the precursor of a blood protease whose target is fibrin. Apo(a) competes with plasminigen for the binding of plasminogen activators. Further more, Lp(a) found within atherosclerotic plaque may contribute to cholesterol ester accumulation within plaque (34). Lp(a) is lipoprotein partially consisting of LDL-c molecule in which the major apolipoprotein of LDL-c (ApoB-100) is linked to an additional large molecule designated Apo(a)(35). The evidence that Lp(a) is a risk factor for CAD events is based largely on retrospective observation studies (36).

Ridker et al (37) were unable to demonstrate an association between Lp(a) and risk of Myocardial Infarction among men, whereas another study supported the association between Lp(a) and coronary disease events(38). In our study, most patients were in relatively younger age group and there were increased levels of plasma ApoB, Lp(a) and LDL-c. These are considered to be the prevalent risk factors in younger group under 40 years of age. As the sample size in our study is smaller, more data will be required to substantiate this finding. Similar finding was reported by Resseneu et al (36) that significantly higher levels of ApoB and Lp(a) in young adults and in middle age men is an independent risk factor for CAD. In diabetic patients the value of triacylglycerols was found to be significantly raised while HDL-c level was significantly lower. Numerous epidemiological studies form North America, Europe and Asia have reported that elevated HDL-c level protects against the CAD, and decreased serum concentration of HDL-c increases the risk of coronary artery disease. The role of triglycerides predicting CAD has been debated for many years and it remain controversial due to highly conflicting findings (37). Present study suggests that high serum triglycerides and decreased HDL-c could be used as maker of CAD. Similar findings were observed by Castell and Catelli (37) who reported positive association of high serum triglyceride concentration and low HDL-c with risk of developing CAD.

CONCLUSION:

CAD patients had significantly lower HDLc and ApoA while Lp(a), ApoB, TAG and LDLc to HDLc ratio were significantly higher in this group. No significant difference was noted in total cholesterol, LDLc and total cholesterol to HDLc ratio between controls and CAD patients.

On subgroup analysis in CAD patients, total cholesterol was markedly lower in diabetics compared with non-diabetic. Higher levels of TAG, ApoB and lower values of LDLc, Lp(a), HDLc were observed in diabetic subgroup .

As a whole, total cholesterol, LDLc and total cholesterol to HDLc ratio were not statistically different among three subgroups viz control, diabetic and non-diabetic CAD patients.

Odd ratio for having CAD was noted to be greater with sedentary life, lower HDLc, higher levels of TAG, Lp(a), ApoB and older age.

Findings in this study have important preventive, diagnostic and therapeutic implications for CAD patients.

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