Serum high-sensitivity C-reactive Protein and Lipoprotein(a) Levels: A comparison between Diabetic and Non-diabetic Patients with Coronary Artery Disease

H Syed Shahid*, M I Kurdi, A A Zohair*****

*Department of Physiology, **Department of Cardiology, ***Department of Emergency Medicine, College of Medicine & King Khalid University Hospital, King Saud University

SUMMARY

Objective: The aim of this study was to compare high-sensitivity C-reactive protein (hsCRP) and Lipoprotein(a) levels [Lp(a)] levels between diabetic and non-diabetic patients with coronary artery disease (CAD). Study Design: Cross sectional Study. Place and Duration of study: This study was conducted in the department of Physiology of College of Medicine & King Khalid University Hospital, King Saud University, Riyadh between August 2006 and December 2007. Methods: One hundred and three individuals with CAD and 30 healthy individuals matched for age and BMI were studied. CAD patients were divided into two groups based on presence (n=62) and absence (n=41) of type 2 diabetes mellitus. Overnight fasting blood samples were collected, and analyzed for total cholesterol (TC), Triglycerides (TG), Low density Lipoprotein (LDL) and High density lipoprotein (HDL), Lp(a) and hsCRP. Data about CAD severity was obtained from medical records. Results: Both groups of CAD without and with DM had significantly higher levels of Lp(a) [mg/dl] (25.58 ± 25.99, 25.90 ± 24.67 respectively) and hsCRP [mg/dl] (0.52 ± 0.71, 0.82 ± 0.78 respectively) when compared with healthy control subjects (Lp(a) =16.93 ± 15.34 & hsCRP=0.27 ± 0.21) [p<0.05]. Lp(a) levels between the two CAD groups were non significant. While, hsCRP levels were significantly high in CAD with DM compared to those without DM [p<0.05]. Gensini Score of CAD severity was also higher in CAD with DM [67.60 ± 45.94] than those without DM [52.05 ± 42.27, p<0.05]. Conclusion: Elevated Lp(a) and hsCRP levels are associated specifically with angiographically defined CAD. However, hsCRP elevation but not Lp(a) is also associated with CAD in type 2 diabetes mellitus. Measurement of hsCRP and Lp(a) may be considered optional markers for better prediction of cardiovascular risk.

KEY WORDS:
Coronary artery disease, Diabetes mellitus, Lipoprotein(a), High-sensitivity C-reactive protein

INTRODUCTION

Several mechanisms linking Lipoprotein(a) [Lp(a)] and development of atherosclerosis have been proposed. In arterial intima, Lp (a) is located only in atherosclerotic plaques, but not in the intact tissue. Lp (a) captured in the atherosclerotic plaque stimulates smooth muscle cells proliferation and its binding to extracellular matrix enhances lipid accumulation. As a non-functional structural homologue of plasminogen it can also negatively affect the process of fibrinolysis [1,2]. There is a significant correlation between the plasma concentration of Lp(a) and the extent of coronary artery disease [3].

More than 20 large prospective trials have shown that the inflammatory biomarker high-sensitivity C-reactive protein (hsCRP) is an independent predictor of future cardiovascular events plus it predicts risk of incident hypertension and diabetes [4]. In type 1 and type 2 diabetes mellitus, hemoglobin A1c significantly correlates with hsCRP levels and future cardiovascular risk. Also, hsCRP levels increase with the stage of beta-cell dysfunction and insulin resistance [5]. Serum glycated albumin and hs-CRP levels were significantly elevated and were independent predictors of CAD in patients with type 2 diabetes and CAD [6]. Although relationship between diabetic atherogenesis and several common risk factors plus non traditional risk markers have been studied extensively and the data is having some controversies, We aimed to study an integrated approach to compare the high sensitivity CRP and Lp(a) in patients with established CAD with and without DM. Therefore we compared High-sensitivity C-reactive protein and Lipoprotein(a) levels between diabetic and non-diabetic patients with stable coronary artery disease.

MATERIALS AND METHODS

This cross sectional study was conducted at the department of Physiology and Cardiology of College of Medicine & King Khalid University Hospital, King Saud University, Riyadh between August 2006 and December 2007. One hundred and three individuals with established CAD and thirty healthy individuals matched for age, sex and BMI were studied. CAD patients were divided into two groups based on presence and absence of DM. Data about CAD severity was obtained from medical records.

A clinical record of each individual including personal data, demographic data, family history and result of the coronary angiography was filled in a predesigned proforma.

Inclusion criteria included patients with established CAD. Exclusion criteria included metabolic diseases like nephrotic

This article was accepted: 11 May 2011
Corresponding Author: Syed Shahid Habib, Department of Physiology (29), College of Medicine, PO Box 2925, King Saud University, Riyadh 11461, Kingdom of Saudi Arabia Email: shahidhabib44@hotmail.com

syndrome, acute or chronic renal failure, thyroid disorders, acute infections, stroke, diabetic ketoacidosis and non-ketotic hyperosmolar diabetes. The medication history was recorded, and the patients taking oral contraceptives and steroids were also excluded. Patients with history of myocrardial infarction in the last two months were also excluded from the study. Subjects who were critically ill or with ongoing or recent (< 1 month) infectious diseases as well as patients with surgical procedure in last 3 months were excluded. The results of patients with hsCRP values >10 mg/L was discarded and were re evaluated after 2-3 weeks. We followed the guidelines of the American Heart Association for measurement, evaluation and expression of hsCRP [7]. Control subjects were free of clinical manifestations of coronary, peripheral or cerebral artery disease by history, physical examination and electrocardiographic findings.

Lipids, High-sensitivity C-reactive protein and Lipoprotein(a) assay

Overnight fasting blood samples were collected, and analyzed for total cholesterol (TC), Triglycerides (TG), Low density Lipoprotein (LDL) and High density lipoprotein (HDL), Lp(a) and hsCRP. TC, TG, LDL and HDL were analyzed by enzymatic colorimetric method with Dimension (USA) kits. hsCRP and Lp(a) were measured by turbidimetric assay with commercial kits (Quantex Lp(a) supplied by BIOKIT, S.A., Barcelona, Spain) on a Hitachi 911, (ROCHE diagnostics, USA). The kit had a working range from 0.10 to 20.0 mg/L for hsCRP. One important attribute of C-reactive protein is its stability over time and the availability of automated assay techniques. Besides, the new assays are very sensitive and provide measurement of C-reactive protein at levels substantially below those levels measured by other traditional methods. For Lp(a) the Limit of Quantification (LOQ) was 1.3 mg/dL and the Limit of Detection (LOD) was 0.4 mg/dL. The autoanalyser used was Hitachi 911, manufactured by ROCHE diagnostics, USA.

Evidence of CAD presence was defined on the basis of at least 50% stenosis in a major coronary vessel. Gensini scoring system was used to determine the CAD severity by assessing luminal narrowing and localization [8]. Multiple lesions in the same vessel were regarded as one-vessel disease.

Statistical Analysis

The data was analyzed by computer software program Statistical Package for Social Sciences (SPSS version 10, Chicago). Descriptive characteristics and lipid profile of the study patients were calculated as Mean ± SD (Standard Deviation) for continuous variables and as percentages for categorical variables. To assess differences in Age, blood pressure, TC, LDL, HDL, TG and BMI analysis of variance was used. Student's t test was used to compare echocardiographic parameters between two CAD groups. Lp(a), CRP and Gensini scoring data because of their extreme skewness, was analyzed by non parametric statistical test Mann-Whitney U test and Wilcoxon (Kruskal–Wallis) test when comparing two or three groups, respectively. A p value of <0.05 was considered as statistically significant. Categorical variables were compared between various groups using Chi square test.

RESULTS

Table I shows comparison of clinical characteristics, lipid profile, Lp(a) and hsCRP between healthy control subjects, CAD patients without DM and CAD patients with DM. Age and BMI matching of CAD patients with control had been difficult. The reason being that CAD patients in the two groups differed significantly in BMI and age. Those without DM were significantly younger and more obese than CAD patients with DM ($p<0.05$). Both groups of CAD patients had significantly higher levels of Lp(a) and hs CRP when compared with healthy control subjects [$p<0.05$]. But when Lp(a) levels were compared between the two CAD groups we observed that the difference was non significant. In case of hsCRP the levels were significantly higher in CAD with DM compared to those without DM [$p<0.05$]. It shows that presence of DM incurs an increased risk of CAD through inflammatory processes also. Gensini Score of CAD severity was also higher in CAD with DM than those without DM [$p<0.05$] [Table II]. The relative percentage distribution of CAD patients in different groups according to number of coronary vessels involved (Coronary vessel score) was determined.I It was observed that diabetic patients with CAD had more diffuse involvement of coronary vessels compared to CAD patients without DM [$p<0.01$] [Figure 1].

DISCUSSION

Our report shows that diabetic patients with CAD have higher CRP levels than CAD patients without DM. While the difference in Lp(a) levels between two groups was non significant. It shows that presence of DM incurs an additional increased CAD risk through inflammatory processes also. This observation is supported by the fact that elevated hs-CRP levels have been associated with other indicators of diabetes-related cardiovascular risk, but have no correlation with disease duration or glucose control [9 20]. Diabetic patients at intermediate or high risk of CAD may benefit from measurement of hs-CRP with regard to individual risk prediction [10 21]. The predictive value of CRP for cardiovascular events and death has been reported to be very high than traditional risk factors or parameters of metabolic control in type 2 diabetic patients.

Recent studies report an independent association of Lp(a) with CAD and DM. In linear regression analysis, LDL-C (or apoB), diabetes, physical inactivity and phosphate are major independent determinants of Lp(a) values. In multiple logistic regression analysis, after adjusting for major risk factors, Lp(a) shows a significant and independent association with the prevalence of CAD [11,12]. O'Brien et al reported that subjects with NIDDM had significantly higher Lp(a) levels than did control subjects, but subjects with NIDDM and CAD did not have significantly higher Lp(a) levels than did those without CAD. Our results are in conformity with their study [13]. There are published controversial data in Lp(a) association with CAD presence and severity. Some studies have observed no association between Lp(a) levels and CAD in diabetic patients [14]. Other researchers reported even lower Lp(a) levels in in diabetic coronary patients and reported no association with the incidence of vascular events. Therefore, although measurement of Lp(a) provides useful information in

Table I: clinical characteristics, lipid profile, Lp(a) and hsCRP between healthy control subjects, CAD patients without DM and CAD patients with DM

	Control N= 30	CAD Without DM N= 41	CAD with DM N= 62
Age	48.83 ± 8.48	49.30 ± 13.24 #	57.63 ± 11.46
BMI	24.82 ± 2.89	26.96 ± 9.32 #	25.54 ± 9.98
SBP	125.67 ± 8.38	129.61 ± 20.37	132.82 ± 20.89
DBP	77.83 ± 6.52	78.61 ± 12.39	76.02 ± 15.47
TC	4.40 ± 0.60	4.27 ± 1.18	4.40 ± 1.38
TG	1.27 ± 0.44*	1.78 ± 0.84	1.77 ± 1.11
LDL	2.74 ± 0.54	2.81 ± 1.10	2.80 ± 1.12
HDL	1.16 ± 0.22*	0.76 ± 0.23	0.75 ± 0.23
Lp(a)	16.93 ±15.34*	25.58 ± 25.99	25.90 ± 24.67
hs CRP	0.27 ± 0.21*	0.52 ± 0.71#	0.82 ± 0.78

Systolic blood pressure (SBP), Diastolic Blood pressure (DBP), Total cholesterol (TC), Triglycerides (TG), Low density Lipoprotein (LDL) and High density lipoprotein (HDL) and Lipoprotein(a) [Lp(a)]. Differences were studied by Wilcoxan (Kruskall Wallis) for Lp(a) & hsCRP and Student's t test for other parameters.
*p<0.05 compared to both CAD groups
# p<0.05 compared to DM with CAD

Table II: Ecocardiographic findings and CAD severity in CAD without DM and CAD with DM

CAD Without	DM N= 41	CAD with DM N= 62
ARD	29.74 ± 3.11	28.84 ± 5.03
LAD	37.13 ± 5.63	38.65 ± 4.88
LVIDd	53.65 ± 10.45	51.82 ± 5.80
LVIDs	37.30 ± 11.54	34.77 ± 7.32
PW	10.17 ± 1.87	10.07 ± 1.65
IVSep	10.87 ± 2.05	10.48 ± 2.04
EF	39.57 ± 15.51	40.84 ± 11.66
Gensini Score	52.05 ± 42.27#	67.60 ± 45.94

Data is expressed as Mean ± SD
Differences were studied by Student's t test
# p<0.05 compared to DM with CAD
Abbreviations: left atrial dimension (LAD), aortic root dimension (ARD), left ventricular internal diameter in diastole (LVIDd), left ventricular internal diameter in systole (LVIDs), left ventricular posterior wall thickness (PW), interventricular septal thickness (IVS),

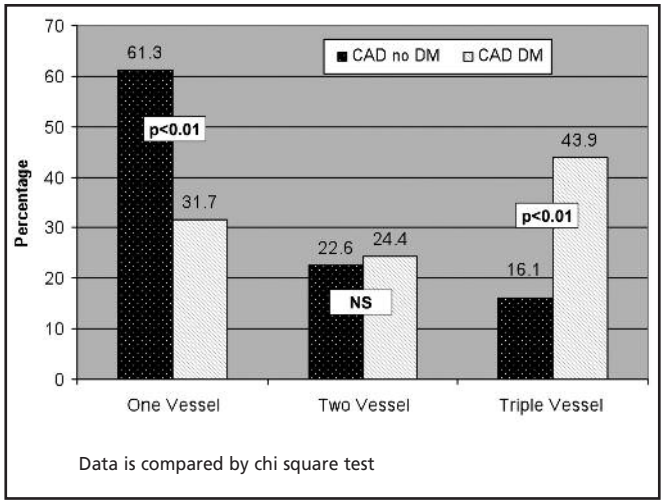

Fig. 1: Percentage distribution of coronary vessel involvement in CAD patients with and without DM.

nondiabetic coronary patients, it is of little value in coronary patients with T2DM [15]. A report from India stated an independent association of Lp(a) with CAD in type 2 diabetic patients. However, they studied healthy subjects, type 2 diabetic patients without and with CAD which is different than our groups which include CAD patients with and without type 2 DM [16]. A long-term predictive value of elevated hsCRP levels has been found in patients with documented coronary artery disease and angina [17,18] and in individuals with multiple risk factors [19]. In type 2 diabetes mellitus, CRP in particular may play a significant role as it amplifies the inflammatory response by stimulating the production of TNF-α and IL-1 by tissue macrophages [20].

Similar to our results Gazzarusoa et al reported that both Lp(a) levels and apo(a) phenotypes may be used not only as predictors of CAD, but also as reliable predictors of CAD severity in type 2 diabetic patients. Indeed, it is possible to hypothesise that diabetic subjects with a genetic predisposition to CAD due to Lp(a) and apo(a) polymorphism may have an earlier and more accelerated coronary atherosclerosis [21].

In current strategies of global risk assessment, lipid testing is the only blood test routinely recommended. However, hsCRP evaluation may have the potential to improve cardiovascular risk prediction models when used as in addition to traditional lipid profiles [22]. In a prospective cohort of women [23], levels of homocysteine, lipoprotein(a), several inflammatory parameters including hsCRP, and a full lipid panel were simultaneously measured as markers of subsequent vascular risk. hsCRP was the single strongest predictor of risk. In multivariate analysis, only hsCRP level and total:HDL

cholesterol ratio prove to have independent predictive value when age, smoking status, obesity, hypertension, family history, and diabetes are also controlled. hsCRP are significantly lowered by hypolipidemic therapy in contrast to Lp(a) which is resistant to conventional treatments.

A limitation of our study is that age and BMI matching of CAD patients with control subjects had been difficult because of significant difference in age and BMI of two groups of CAD patients. CAD patients without DM were significantly younger and more obese than CAD patients with DM. The reason can be that obesity and physical inactivity are risk factors for CAD. Obesity is associated directly with increased plasma levels of hsCRP, an observation consistent with findings that adipocytes secrete interleukin-6, a primary hepatic stimulant for CRP production [24,25].

CONCLUSION

Patients with CAD have higher levels of hsCRP and Lp(a) than healthy individuals. Diabetic patients with CAD have significantly higher hsCRP levels as compared to non-diabetic patients. But this difference is not significant for Lp(a) levels. These data indicate that elevated Lp(a) and hsCRP levels are associated specifically with angiographically defined CAD. However, hsCRP elevation but not Lp(a) is also associated with CAD in type 2 diabetes mellitus. Measurement of hsCRP and Lp(a) may be considered optional markers for better prediction of cardiovascular risk.

ACKNOWLEDGEMENT

We are thankful to the College of Medicine Research Center for supporting this study.

REFERENCES

1. Koschinky ML, Marcovina SM: Structure-function relationships in apolipoprtein (a): insights into lipoprotein (a) assembly and pathogenicity. Curr. Opin. in Lipidol 2004; 15(2): 167-74.
2. Lippi G, Guidi G: Lipoprotein(a): An emerging Cardiovascular Risk Factor. Crit. Rev. Clin. Lab. Sci. 2003; 40(1): 1-42.
3. Dangas G, Ambrose JA, D'agate DJ: Correlation of plasma lipoprotein(a) with the angiographic and clinical presentation of coronary artery disease. Am J Cardiol 1999; 83: 583-5.
4. Ridker PM. C-reactive protein and the prediction of cardiovascular events among those at intermediate risk: moving an inflammatory hypothesis toward consensus. J Am Coll Cardiol. 2007; 49(21): 2129-38.
5. Pfutzner A, Forst T: High-sensitivity C-reactive protein as cardiovascular risk marker in patients with diabetes mellitus. Diabetes Technol Ther 2006, 8: 28-36.
6. Pu LJ, Lu L, Xu XW, *et al*. Value of serum glycated albumin and high-sensitivity C-reactive protein levels in the prediction of presence of coronary artery disease in patients with type 2 diabetes. Cardiovasc Diabetol. 2006 Dec 20; 5: 27.
7. Thomas A. Pearson TA, Mensah GA, *et al*. Markers of Inflammation and Cardiovascular Disease. Application to Clinical and Public Health Practice. Circulation. 2003; 107: 499-511.
8. Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. Am J Cardio. 1983; 51: 606.
9. Linnemann B, Voigt W, Nobel W, Janka HU. C-reactive protein is a strong independent predictor of death in type 2 diabetes: association with multiple facets of the metabolic syndrome. Exp Clin Endocrinol Diabetes. 2006 Mar; 114(3): 127-34.
10. Schulze MB, Rimm EB, Li T, Rifai N, Stampfer MJ, Hu FB.C-reactive protein and incident cardiovascular events among men with diabetes. Diabetes Care. 2004 Apr; 27(4): 889-94.
11. Rasoulii M, Kiasari AM. Interactions of lipoprotein(a) with diabetes mellitus, apolipoprotein B and cholesterol enhance the prognostic values for coronary artery disease. Clin Chem Lab Med. 2008; 46(5): 667-73.
12. Rasouli M, Kiasari AM, Mokhberi V. The ratio of apoB/apoAI, apoB and lipoprotein(a) are the best predictors of stable coronary artery disease. Clin Chem Lab Med. 2006; 44(8): 1015-21.
13. O'Brien T, Nguyen TT, Harrison JM, Bailey KR, Dyck PJ, Kottke BA: Lipids and Lp(a) lipoprotein levels and coronary artery disease in subjects with non-insulin-dependent diabetes mellitus. Mayo Clin Proc 1994; 69: 430-5.
14. Pedreno J, Fernandez R, Ballester A, *et al*. Lack of association of serum lipoprotein (a) levels with type-2 diabetes mellitus in patients with angiographically defined coronary artery disease. Int J Cardiol 2000; 74: 159-67.
15. Saely CH, Koch L, Schmid F, *et al*. Lipoprotein(a), type 2 diabetes and vascular risk in coronary patients. Eur J Clin Invest 2006; 36: 91-7.
16. Mohan V, Deepa R, Haranath SP, *et al*. Lipoprotein(a) is an independent risk factor for coronary artery disease in NIDDM patients in South India. Diabetes Care 1998; 21: 1819-23.
17. Thompson SG, Kienast J, Pyke SDM, Haverkate F, van de Loo JCW. Hemostatic factors and the risk of myocardial infarction or sudden death in patients with angina pectoris. N Engl J Med 1995; 332: 635-41.
18. Haverkate F, Thompson SG, Pyke SDM, Gallimore JR, Pepys MB, for the European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study Group. Production of C-reactive protein and risk of coronary events in stable and unstable angina. Lancet 1997; 349: 462-6.
19. Kuller LH, Tracy RP, Shaten J, Meilahn EN, for the MRFIT Research Group. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Am J Epidemiol 1996; 144: 537-47.
20. Nesto R. C-reactive protein, its role in inflammation, Type 2 diabetes and cardiovascular disease, and the effects of insulin-sensitizing treatment with thiazolidinediones. Diabet Med 2004; 21: 810-7.
21. Gazzaruso C, Bruno R, Pujia A, *et al*. Lipoprotein(a), apolipoprotein(a) polymorphism and coronary atherosclerosis severity in type 2 diabetic patients. International Journal of Cardiology 2006; 108 (3): 354-58.
22. Ridker PM, Glynn RJ, Hennekens CH. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. Circulation. 1998; 97: 2007-11.
23. Ridker PM. High-Sensitivity C-Reactive Protein Potential Adjunct for Global Risk Assessment in the Primary Prevention of Cardiovascular Disease. Circulation. 2001; 103: 1813-18.
24. Visser M, Bouter LM, McQuillen GM, *et al*. Elevated C-reactive protein levels in overweight and obese adults. JAMA. 1999; 282: 2131-35.
25. Yudkin JS, Stehouwer CDA, Emeis JJ, *et al*. C-reactive protein in healthy subjects: associations with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? Arterioscler Thromb Vasc Biol. 1999; 19: 972-78.