Original Research Article

International Journal of Bioassays
ISSN: 2278-778X
www.ijbio.com
OPEN ACCESS

ASSESSMENT OF LIPOPROTEIN-ASSOCIATED PHOSPHOLIPASE A2 IN CORONARY ARTERY DISEASE PATIENTS OF NORTH INDIA

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Received for publication: May 17, 2014; **Revised**: June 21, 2014; **Accepted**: July 07, 2014

Abstract: Lipoprotein associated phospholipase A2 (Lp-PLA2) is an inflammatory biomarker which play an important role in the pathophysiology of coronary artery disease (CAD). Lp-PLA2 is produced by inflammatory cells like monocytes, macrophage etc. it hydrolyses platelet activating factor and oxidatively fragmented phospholipids. It is an emerging risk factor for CAD, because of its high vascular specificity and direct relation with plaque instability and rupture. Several reports reveal that plasma Lp-PLA2 is a good biomarker of vascular inflammation, atherosclerotic vulnerability and future cardiovascular events. The correlation between Lp-PLA2 and CAD remains poorly investigated in north Indian population. This study is aimed to investigate the Lp-PLA2 levels as a predictor of CAD. Study was conducted with One hundred fifty CAD patients and One hundred fifty normal healthy subjects. Serum Lp-PLA2 level estimation reveal that CAD patients bears significantly elevated circulatory Lp-PLA2 levels in comparison to normal healthy population. The increase in Lp-PLA2 levels was found irrespective of sex i.e., male and female. We also observed a non-significant change in Lp-PLA2 levels among CAD male and female patients. The study suggests that Lp-PLA2 may be proatherogenic and contribute to atherogenicity and incidence of cardiovascular disease. The stimulated activity of this enzyme may predict CAD patients without known CAD, independent of heart disease risk factors. We can conclude that there is a positive correlation between Lp-PLA2 and risk of CAD events and hence Lp-PLA2 can be considered as a risk associated inflammatory marker for CAD, in north India population.

Key Words: Lp-PLA2, Coronary artery disease, risk factor, Biomarker, north India.

INTRODUCTION

Atherosclerosis (Arteriosclerosis Vascular Disease or ASVD) is of major concern globally which can led to increased risk in mortality and morbidity. Among these, coronary artery disease (CAD) is a common manifestation of deposition of atherosclerotic plaque in side arteries that may coexists with conditions like hypertension, dyslipidemia, chest-pain and ischemic heart. Furthermore, the association of inflammatory molecules in CAD patients may play an important role in progression in atherogenesis, thrombosis, plaque vulnerability and rapture causing angina pectoris, myocardial infraction and heart stroke [1]. Inflammation plays an important role in atherosclerosis and its clinical implications such as myocardial infarction and stroke [2]. Several serum inflammatory markers are associated with carotid athersosclerosis [3]. Among the serum markers of inflammation (fibrinogen, CRP, Lp(a), microalbumin, ceruloplasmin PAI-1, IL-6 and amyeloid–A) known to be associated with atherosclerosis and CVD, the increased level of enzyme Lipoprotein-associated Phospholipase A2 (Lp-PLA2) is correlated with the progression, extension, and severity of CVD as well as to a greater mortality risk after coronary heart attack [4].

Lp-PLA2, also known as platelet activating factors acetylhydrolase (PAF-AH), is a 50 KDa ca^{2+} independent enzyme synthesized by macrophages and platelets [5]. The enzyme is carried in the blood stream by low density lipoprotein (LDL) particles. The majority of LDL-associated Lp-PLA2 (LDL-Lp-PLA2) is bound to atherogenic small-dense LDL (sd-LDL) particles [6]. Lp-PLA2 is considered as a proatherogenic enzyme, as it causes hydrolysis of modified phospholipids within oxidized LDL(Ox-LDL) generates pro-inflammatory oxidized non-etherified fatty acids and lysophosphatidylcholine which are involved in various stages of atherosclerotic plaque development and may also play an important role in plaque vulnerability [7]. This enzyme is expressed in atherosclerotic plaques and within fibrous cap of human rupture prone lesions by inflammatory cells; thus, its measurement could contribute to the identification of patients with rupture prone plaques [8]. In fact, Lp-PLA2 has been recommended as an inflammatory marker for CVD risk assessment in patients with moderate or high risk based on Framingham criteria [9]. Plasma levels of this enzyme vary from species to species (mice and human), the reason is not known for this difference [10].

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Genetic studies demonstrate the inter-individual variation of Lp-PLA2 activity [11]. A recent study has reported that oxidized LDL and its unhydrolysed oxidized phospholipids can increase Lp-PLA2 expression in monocytes [12]. Lp-PLA2 is also responsible for the release of atherogenic isoprostanes from esterified phospholipids which are involved in inflammation and atherosclerosis [13]. Lp-PLA2 is associated with the progression of CAD and has been predicted as marker of cardiovascular events, therefore it is considered as a therapeutic target in post-transplant patients [14]. Several epidemiologic studies have also suggested that plasma Lp-PLA2 levels may be considered as a biomarker and an independent predictor of cardiovascular events in primary and secondary prevention [15].

It is important to identify patients at risk of CAD for clinical management, treatment and prognosis. Because conventional risk factors do not explain the changes in atherosclerosis, efforts to identify vulnerable plaques have focused on establishing novel biomarker. To our knowledge, there has been no study available, which has evaluated Lp-PLA2 as a prognostic marker after CAD. We planned this study with a hypothesis that comparative evaluation of Lp-PLA2 levels at the time of first CAD event is associated with increased risk of recurrent event.

MATERIAL AND METHODS

Study place, design and patient recruitment

The study was conducted between Feb 2013 and March 2014. Total 300 participants, with their ages ranging from 40 to 60 years, attending IPD and OPD of Department of Medicine, Era's Lucknow Medical College & Hospital, Lucknow, were recruited for this cross-sectional study. The study was conducted after obtaining approval from Institutional Ethical Committee and obtaining the informed consent from the patients in vernacular language. Among these 300 participants, 150 were clinically newly diagnosed case of CAD like angina pectoris and myocardial infarction. Control group consist of 150 healthy individuals with no known history of any disease. All the patients were examined clinically and information pertaining to age, gender, habits, high blood pressure, smoking and health status was recorded in patient information sheet. The patients were diagnosed with chronic stable angina and with acute coronary syndrome i.e. unstable angina. Participants were considered as hypertensive if they had a blood pressure > 140/90 mmHg on two or more occasions or were already on antihypertensive therapy. If the patients had been given lipid lowering therapy or had a history of total cholesterol levels >240 mg/dl, they were accepted as hyperlipidemic.

Exclusion criteria

Patients with chronic liver and renal diseases, rheumatoid arthritis, hormonal imbalances, those who were taking steroidal hormone, alcohol, oral contraceptive drug and other medication contradictive to CVD, as well as pregnant women participants were excluded from the study.

Anthropometric measurements

Various anthropometric measurements (including height, weight, and waist circumference) were taken as per standard protocol. Waist circumference was measured with a flexible soft measuring tape at the level of umbilicus (midway between the lowest rib and iliac crest), at the end of a relaxed expiration in standing position. Hip circumference (cm) was measured using the point of measurements, i.e. height (without shoes) and weight (with heavy clothing removed). BMI was calculated as a ratio of weight (kg) to height (meter). BMI cut offs were classified in accordance with the revised Indian guidelines for BMI by Union Health Ministry of India (2005) : Less than 18.4 Kg/m^2 (underweight), 18.5-22.9 Kg/m^2 (normal), 23-24.9 Kg/m^2 (overweight) and >25 Kg/m^2 (obese).

Sample collection and biochemical analysis

After obtaining the consent, 5ml blood was drawn from healthy group and CAD patients by venipuncture in plain vial. The blood was allowed to clot and centrifuged at 2000 rpm for 15 minutes at room temperature. Serum were collected and kept at -20°C until the test was performed.

Lp-PLA2 estimation

Lp-PLA2 estimation was done using the ELISA kit, procured from RayBiotech Inc, USA. The test was performed in serum using a double antibody sandwich ELISA. The technical bulletin supplied along with the ELISA kit was adopted. The assay range of the kit was 9.3-300ng/ml.

Statistical analysis

One-way-analysis of variance (ANOVA test) was performed by comparison of values for patient group with control. All hypothesis testing were two-tailed. $P<0.05$ was considered statistically significant and the results were expressed as mean ± SD. The Graph pad INSTAT 3.0 software was used to carried out the statistical analysis.

RESULTS AND DISCUSSION

We recruited 150 normal healthy individual among which 105 were males and 45 were females, while among 150 CAD patients, 122 were males and 28 were females. All the CAD patients were newly diagnosed cases by cardiologist of our institute. 68.33%

of the patients were in the age group 48 to 60 years, and 31.33% were in the age group 40 to 48 years. Upon BMI analysis, we recorded 52% patients as obese, 39% as overweight and rest lie among the normal values. Blood pressure investigation revealed 43.33% patients as hypertensive, 37.33% patients as pre-hypertensive and 19.33% showed normal value. Only 10% patients had family history of CAD or hypertension. 68.66% patients were non-smokers, only 31.33% patients reported to have occasional smoking habit.

In the present study, the patient having chest pain and CVD like symptoms were investigated. Lp-PLA2 was analysed in the serum of all study participants. Results are illustrated in table 1. Our investigation reveals that Lp-PLA2 levels in normal healthy individual were 28.50±5.41 and 31.72±6.83 for male and female, respectively. Values are expressed in ng/ml and as mean±SD. The Lp-PLA2 levels ranged between 17.12 to 32.43 ng/ml in males and 19.21 to 36.68 ng/ml. Among the hospitalized CAD patients, blood was drawn within 72 hours of cardiovascular event (CV). Among CAD patients, 81.33% were males and 18.66% were females. Lp-PLA2 levels in CAD patients were 39.34±10.37 and 43.22±7.62 ng/ml for males and females respectively. The range of Lp-PLA2 in male patients was 26.38 to 50.16 ng/ml and 33.18 to 51.82 ng/ml. When we statistically analysed our results, we found a significant ($p<0.05$) increase in Lp-PLA2 levels in CAD patients as compared to normal healthy individuals. This change was recorded statistically significant. We observed the higher levels of Lp-PLA2 as 50.16 and 51.82 ng/ml among male and female CAD patients respectively. When compared for intra group statistics, non-significant change was observed, among CAD patients. This reveals that Lp-PLA2 is independent value for both sex, and is not the characteristic of sex. Percentage increase change for Lp-PLA2 levels in normal and CAD male patients was 38.03%, while for females this increase was 36.25%. This change indicates the elevation of Lp-PLA2 levels in CAD patients.

Table 1: Comparison of Lp-PLA2 levels (ng/ml) in control and CAD patients.

Gender	Control (n= 150)	CAD Patients (n=150)	Change (in percentage)
Males	28.50 ± 5.41	39.34 ± 10.37*	+38.03
Females	31.72 ± 6.83	43.22 ± 7.62*	+36.25

Values are expressed as mean ± SD
CAD Patient group was compared with control.
*P<0.05 considered as significant

Lp-PLA2 has been associated with increased risk of incident ischemic cardiac and CV event in several epidemiologic studies [16,17]. The 2010 ACCF/AHA Guidelines for Assessment of Cardiovascular Risk in Asymptomatic Adults recommend Lp-PLA2 as a reasonable inflammation serum marker for CVD risk assessment in intermediate risk asymptomatic adults, however in a study; men and women with metabolic syndrome had shown a weak direct correlation between Lp-PLA2 levels [18]. In our study, male gender was predominant in this investigation. The participants of patient group also had some factors of metabolic syndrome, like obesity and hypertension following mild dyslipidemia: all these factors are known to initiate CVD. Many experimental and epidemiological studies have shown that high levels of Lp-PLA2 are associated with an increased risk for initial coronary events, recurrent coronary events [19]. Lp-PLA-2 participates directly in atherogenesis by potentiating lipid modification and inflammation. Lp-PLA-2 hydrolyzes phosphatidycholine to form lysophosphatidycholine and oxidize free fatty acids, both of which stimulate artherosclerosis. Within the individual, serum level of this enzyme have low bio variability and reflect the presence of rupture prone atherosclerotic plaques in both men and women.

To our knowledge, no previous data are available on the relationship of Lp-PLA2 to outcome after CV event. The present study provides a strong evidence that Lp-PLA2 may be associated with risk of recurrent CV event after first one. Further it also indicates that Lp-PLA2 may provide a more specific marker of the risk associated with vascular disease as compared to other conventional inflammatory markers.

CONCLUSION

We can conclude from present study, there is a close relation between Lp-PLA2 and prognosis after CV event. The marker may provide other complementary information about the risk of recurrent stroke and other vascular event. Further, a large population based cohort study is needed to establish Lp-PLA2 as predictor and prognosis marker for CV event in north Indian population.

ACKNOWLEDGEMENTS

The authors are thankful to Prof. Ajanta Roy, Head, Department of Biochemistry and Dr. Ramesh Chander, Emeritus scientist, Department of Biochemistry for their time to time valuable suggestions and scientific support.

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Source of support: Nil
Conflict of interest: None Declared