DISCUSSION

Despite great advances in terms of screening, diagnosis, treatment and prevention in the past decades; cardiovascular diseases (CVD) are still the leading cause of morbidity and mortality worldwide.[188] It has been well documented that the initiation and progress of CVD is largely associated with the severity of atherosclerosis. Atherosclerosis is recognized as a chronic and dynamic status of vascular inflammation, and usually has already existed for years or decades before cardiovascular (CV) events occur. [189-191]

It's well known that vascular inflammation, a hall-mark of the beginning of atherosclerosis, is primarily incurred by endothelial dysfunction. Accordingly after being exposed to traditional risk factors for a certain period, such as obesity, smoking, hypertension, dyslipidemia and diabetes mellitus, endothelial cells gradually become dysfunctional and the natural barrier built among endothelia is impaired. Subsequently, lipids begin accumulating in sub-endothelial spaces. Macrophages infiltrate and engulf lipids and turn into foam cells and concomitantly produce inflammatory cytokines, reactive oxygen species and chemotactic factors, which are largely derived from ox LDL hydrolysis. Afterward, more leukocytes infiltrate and accumulate, lipid oxidation aggravates, endothelial cells deteriorate, and consequently vascular inflammation propagates. [192,193,19)]

Lp-PLA_2 also known as platelet-activating factor acetylhydrolase or type VIIA PLA_2, is composed of 441 amino acids. Lp-PLA_2 is a Ca +2 independent phospholipase which belongs to phospholipase A_2 superfamily. [194] In the atherosclerotic plaque, Lp-PLA_2 hydrolyzes oxLDL into Lyso-PC and oxidized non-esterified fatty acids (ox NEFAs), both of which play important and multiple roles in atherogenesis.[195-197] Lp-PLA_2 is a novel and unique biomarker, highly specific for vascular inflammation and atherosclerosis [198,199] that provides information about atherosclerotic plaque inflammation and stability. Elevated levels of serum Lp-PLA_2 are indicative of rupture prone plaque and a strong independent predictor of cardiovascular risk, including coronary artery disease, MI, and stroke; [200,178] So it appears that Lp-PLA_2 is released from these plaques into the circulation. [201]

The present study aimed to determine Lp-PLA_2 level in myocardial infarction patients for the first time in Egyptian population. Thirty consecutively selected Egyptian patients admitted to Cardiology Department at Alexandria Main University Hospital, whose age ranged from 35-61 years with a mean of 48.53 ± 8.14 years. The study included 66.7% males and 33.3% females and suffering from MI which was confirmed by ECG changes and elevation of cardiac enzymes (CK-MB mass and Troponin I). Patients with DM, liver, renal, pituitary or thyroid diseases were excluded to eliminate the relationship between the Lp-PLA_2 and diseases other than CVD.

Also, twenty healthy individuals, whose age ranged from 30-61 years with a mean of 44.85 ± 9.94 years, including 70% males and 30% females with no history of hypertension, DM, atherosclerosis, cancer or medications were included as a control group.

In the present study, detailed history was taken regarding other cardiovascular risk factors such as hypertension and smoking. We found that 43% of our patients were hypertensive and 46.7% were smokers. Fasting blood glucose was measured to exclude

DM of patients and controls. Also, AST, LDH, CK-MB and Tn I were measured to confirm that the patients suffered from ACS. Finally hs-CRP was measured as a marker of inflammation, as Lp-PLA_2.

In the present study, we detected the Lp-PLA_2 activity of patients and controls using enzymatic assay method using Beckman Coulter AU 400 analyzer.

In our study we found significant increase in Lp-PLA_2 activity levels in MI patients when compared to healthy controls.

Oei HH et al. [202] conducted a prospective case cohort study (The Rotterdam Study) including 308 coronary heart disease cases, 110 ischemic stroke cases, and a random sample of 1820 healthy subjects ≥55 years, to investigate whether Lp-PLA_2 is an independent predictor of coronary heart disease and ischemic stroke. He found that Lp-PLA_2 activity is an independent predictor of coronary heart disease and ischemic stroke in the general population independent of classic cardiovascular risk factors and CRP.

Cook NR et al. [203] investigated a case cohort sample from the Women's Health Initiative Observational Study (WHI-OS) comprised 1821 CVD cases and a reference 1992 healthy post-menopausal women with a mean age of 68 years to evaluate the association of Lp-PLA_2 mass and activity with CVD (myocardial infarction, stroke and CVD mortality); found that Lp-PLA_2 activity were correlated with risk of incident CVD, and showed a marginal association with MI.

Winkler K et al. [204] performed a prospective cohort study (The Ludwigshafen Risk and Cardiovascular Health Study; LURIC) in 2513 patients with angiographic confirmed CAD and in 719 patients without angiographic confirmed CAD, to investigate that Lp-PLA_2 activity can predict cardiac mortality in patients scheduled for coronary angiography during median observation period of 5.5 years. He found that Lp-PLA_2 activity predicts risk for 5-year cardiac mortality independently from established risk factors and indicates risk for cardiac death in patients with low and medium-high hs-CRP concentrations.

Rallidis LS.et al. [205] conducted a prospective cohort study LAERTES (Lipoprotein-Associated phospholipase A_2 in stable coronary artery disease study) on 477 patients with stable CAD with exclusion criteria of ACS ,coronary artery bypass grafting within the previous 6 months, clinical evidence of heart failure, chronic renal failure, age>75 years and coexistent neoplasm or inflammatory disease. He found that Lp-PLA_2 activity was an independent predictor of cardiac death after adjustment of conventional risk factors (age, sex, diabetes mellitus, total cholesterol, hypertension, smoking, and family history of CAD).

Jenny NS et al. [206] performed a cohort study on 5888 men and women ≥65 years of age with exclusion of 1565 participants with prevalent CVD to examine associations between Lp-PLA_2 antigen level (mass) and enzymatic activity (activity) and CVD (results from the Cardiovascular Health Study). He found that mean Lp-PLA_2 activity levels were higher in MI ($p<0.001$) and CVD death ($p=0.004$) cases than the event-free group.

Tsimikas S et al. [207] conducted a prospective population-based survey in 765 subjects over 10-year follow-up period to identify factors that influence plasma levels and assess the prognostic value of Lp-PLA_2 activity. He found that increased Lp-PLA_2 activity

was significantly related to incident CVD (P<0.001) and with vascular mortality but not with non-CVD mortality.

Hatoum IJ et al. [208] carried out a prospective cohort study of 740 men and 777 women with diabetes who were free of cancer and cardiovascular disease to assess the association of Lp-PLA_2 with future coronary events among diabetic men and women. He found that Lp-PLA_2 activity was an independent risk factor for coronary heart disease. He also investigated 178 men cases of CHD during 10 years and 146 women cases of CHD during 14 years; he found that Levels of Lp-PLA_2 activity were significantly associated with incident CHD among men and women with type 2 diabetes, independent of traditional and inflammatory risk factors.

Hatoum IJ et al. [209] investigated 121,700 U.S female nurses aged 30–55 years old among participants of the Nurses' Health Study to assess the association between Lp-PLA_2 activity and incident CHD in a large population of disease-free women. He detected 421 cases of incident myocardial infarction (MI) during 14 years of follow-up. He also found that Lp-PLA_2 activity was higher in cases than in controls, and Levels of Lp-PLA_2 activitywere significantly associated with incident CHD among women. In addition, Lp-PLA_2 activity added significantly to CHD risk discrimination.

Maiolino G et al. [210] performed a prospective cohort study of 712 Caucasian patients, who underwent coronary angiography and measurement of both Lp-PLA_2 mass and activity at baseline to investigate chest pain and/or suspected CAD. With exclusion of patients with incomplete follow-up data. Thus, the final analysis was carried out in 506 patients during 7.2 years follow up. He found that a high Lp-PLA_2 activity implies a worse CV prognosis at long term follow up in high-risk Caucasian patients referred for coronary angiography.

Person M et al. [211] carried out a sub-study of the epidemiology of carotid artery disease from (The Malmo Diet and Cancer Study [MDCS]) which is a prospective cohort study examining the association between diet and cancer in 4480 subjects aged 45 to 69 years. The study aimed to explore potential interrelationships between Lp-PLA_2, metabolic syndrome, and incident cardiovascular disease; with exclusion of Subjects with history of MI, stroke, diabetes mellitus and use of anti-diabetic medication. he found that patient with metabolic syndrome had higher levels of Lp-PLA_2 activity during a mean follow-up period of 10.6 years ; 261 developped first CVD events [28 fatal coronary events, 103 non-fatal myocardial infarctions, 101 ischemic, and 29 hemorrhagic strokes], So Lp-PLA_2 activity were associated with incident CVD (p=0.001).

Anuurad E et al. [212] detected 224 African-Americans and 336 Caucasians under-going coronary angiography to investigate the association between Lp-PLA_2 and CAD in a bi-ethnic African-American and Caucasian population. He found that subjects with CAD had significantly higher levels of Lp-PLA_2 activity compared with those without CAD across ethnicity.

Liu CF et al. [213] conducted a prospective study on 146 consecutive patients with CAD who underwent clinically-indicated coronary angiography and pre-interventional intravascular ultrasound (IVUS) to evaluate the relationship between Lp-PLA_2 activity and vulnerable plaque in patients with CAD. He found that Plasma Lp-PLA_2 activity is

associated with plaque rupture in patients with CAD, independently of traditional CAD risk factors.

In the present study there was no statistically significant difference between Lp-PLA_2 activity levels regarding gender (p=0.489).

On the other hand Oei HHet al [202] ,Winkler K et al [204] and Tsimikas S et al [207] found that Lp-PLA_2 activity level was higher in males than females (p<0.001) ; that variation may be due to increase age of our female cases (mean=50.93 ± 8.83) that lead to decrease estrogen protective effect of female patients.

Maiolino G et al. [210] found that gender difference is significant predictor of Lp-PLA_2 activity but it was an inverse correlation (p=0.039) which is not concomitant with our results.

Oei HH et al [202] found that Lp-PLA_2 activity was not significantly correlated with smoking (p=0.37) which agree with our study (p=0.198).

On the other hand Rallidis LS et al. [205] found that Lp-PLA_2 activity was positively correlated with smoking (p<0.028).

In our study there was no statistically significant correlation between Lp-PLA_2 activity levels regarding hypertension (p=0.562).

On the other hand Oei HH et al [202] found that Lp-PLA_2 activity was positively correlated with high systolic blood pressure (p=0.003) and was not significantly correlated with high diastolic blood pressure (p=0.83).

Oei HH et al [202] found that Lp-PLA_2 activity was not significantly correlated with increasing age (p=0.29) which is in accordance with our results (p=0.745).

On the other hand Tsimikas S et al [207] found that Lp-PLA_2 activity level modestly increased with age (P=0.045).

On the other hand Hatoum IJ et al. [208] found that shorter duration of diabetes was associated with higher Lp-PLA_2 activity. That variation from our results (p= 0.714) may be due to exclusion of diabetic patients in our study. (table XII)

In the present study there was no statistically significant correlation between Lp-PLA_2 activity levels regarding hs-CRP (p=0.786).

On the other hand Cook NR et al. [203] found that Lp-PLA_2 activity was correlated with lower hs-CRP (p<0.0001) and Rallidis LS.et al. [205] found that Lp-PLA_2 activity was positively correlated with higher hs-CRP (p<0.001).

Winkler K et al. [204] and Tsimikas S et al. [207] found positive correlation with increasing triglycrides. Similarly Anuurad E et al. [212] found positive correlation with increasing triglycrides (p=0.021 in Caucasians), (p=0.022 in African-Americans) and Hatoum IJ et al. [209] (p<0.001) which is concomitant with our results (p=0.017)

On the other hand Da Silva IT et al. [214] who conducted a cross sectional study on adolescents between ten and nineteen years old to evaluate the influence of obesity and

cardio-metabolic markers on Lp-PLA_2 activity in adolescents. With exclusion of smokers, the use of alcohol, the use of lipid-lowering drugs, the presence of any acute disease, the participation in other protocols research and pregnancy or breastfeeding. He reported that Lp-PLA_2 activity was not correlated with increasing triglycrides.

In our study there was no statistically significant correlation between Lp-PLA_2 activity levels regarding increasing total cholesterol level (p=0.989)

On the other hand Oei HH et al. [202], Tsimikas S et al. [207] , Anuurad E et al. [212] and Hatoum IJ et al. [209] found positive correlation with increasing total cholesterol level (p<0.001). Similarly Cook NR et al. [203] (p<0.0001) and Da Silva IT et al. [214] found positive correlation,

Oei HH et al. [202], Maiolino G et al. [210] , Hatoum IJ et al. [209] and Anuurad E et al. [212] found positive correlation with higher LDL-C level (p<0.001) which is in accordance with our results (p=0.003).

Similarly Winkler K et al. [204] , Hatoum IJ et al. [208] , Da Silva IT et al. [214] and Rallidis LS.et al. [205] (p<0.0001) found positive correlation with increased LDL-C

Oei HH et al. [202] and Hatoum IJ et al. [209] reported an inverse correlation with increased HDL cholesterol (p<0.001) which correlate with our results (p=0.035, r=0.386)

Similarly Tsimikas S et al. [207], Hatoum IJ et al. [9] and Cook NR et al. [203] (p<0.0001) found inverse correlation with increasing HDL-C level.

On the other hand Da Silva IT et al. [214] and Anuurad E et al. [212] (p<0.001 for Caucasians) and (p=0.006 for African-Americans) found no correlation with HDL-C.

In the present study we found that the cutoff value for Lp-PLA_2 activity was 204.6 nmol/min/ml, with 76.67% sensitivity, 75% specificity and 76% accuracy with significant p-value (<0.001).

Maiolino G et al [210] found that Youden index cut-off that best predicted CV events for Lp-PLA_2 activity was 136.1 nmol/ml/min (AUC=0.707, p<0.0001) which is in agreement with our study.

Similarly Tsimikas S et al [207] found that addition of Lp-PLA_2 activity level to equations based on the Framingham risk function (included sex, DM, BMI, smoking, previous CVD, LDL-c and CRP) resulted in a modest increase in the area under the receiver – operating characteristic (ROC) curve for risk prediction (0.737 vs. 0.717, Δ 0.020, P=0.31). (figure 35)

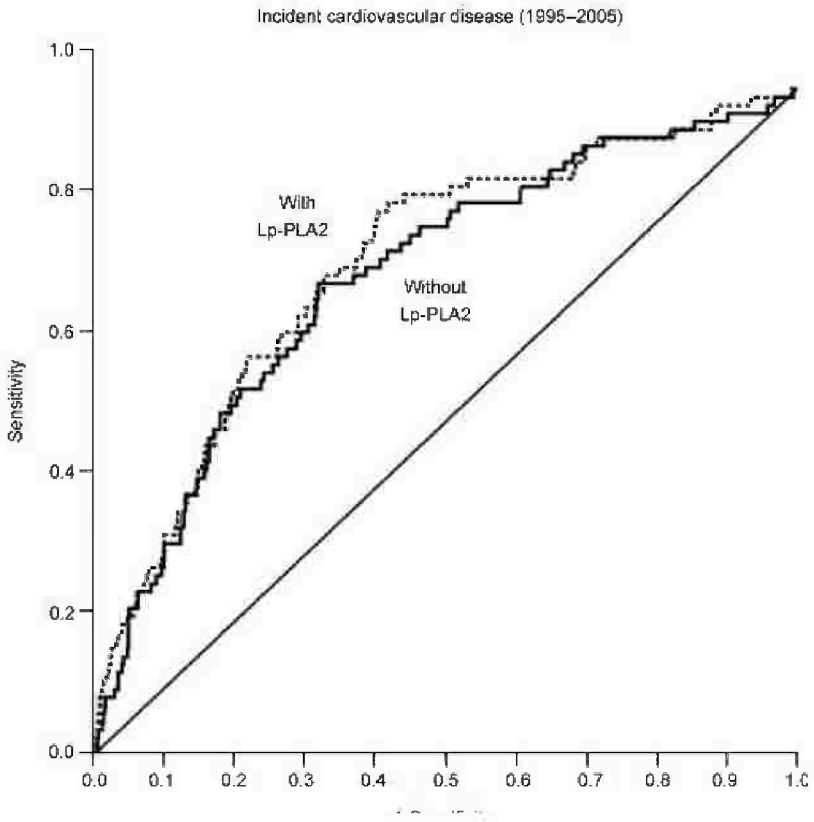

Figure (35): Receiver–operating characteristic (ROC) curves for incident cardiovascular disease (1995 – 2005). Curves are based on models of prediction of risk using conventional risk variables (Framingham Risk Score) with and without the level of Lp-PLA_2 activity.

SUMMARY

CAD is a condition in which plaque builds up inside the coronary arteries. These arteries supply oxygen-rich blood to heart muscle.

CAD is the most common form of cardiovascular disease, whose underlying pathological feature is atherosclerosis. Atherosclerosis is a slowly progressing chronic disease of large and medium-sized arteries which is characterised by the formation of atherosclerotic plaques.

Lp-PLA_2, also known as platelet activating factor acetyl-hydrolase, is a novel biomarker that is secreted from inflammatory cells involved within the atherosclerotic plaque. Hence it is highly specific for vascular inflammation not systemic inflammation.

Lp-PLA_2 enzyme has a broad specificity for oxPLs. This suggests a major specific role of Lp-PLA_2 in the depletion of oxPLs from lipoproteins, with production of Lys-PC and free oxidized fatty acids, which are inflammatory mediators.

So, the present study aimed to determine serum Lp-PLA_2 activity in Egyptian patients having myocardial infarction. This study was conducted on thirty Egyptian patients admitted to Cardiology Department at Alexandria Main University Hospital, including 20 males (66.7%) and 10 females (33.3%), whose age ranged from 35-61 years with a mean of 48 years.

They were suffering from myocardial infarction which was confirmed by ECG changes and elevation of cardiac enzymes (CK-MB and Tn I). Those with diabetes mellitus, liver, renal, and thyroid diseases were excluded to eliminate any relationship between the enzyme activity and diseases other than CAD.

Also, twenty healthy individuals, including 14 males (70%) and 6 females (30%), aged 30-61 years with a mean of 44 with no history of diabetes, hypertension and medications were included as control group.

Five milliliters of venous blood were drawn from every patient on admission and emptied in a plain tube then the separated serum was divided in to two parts, the first of which was analyzed on Dimension RxL Max analyzer (Siemens Healthcare Diagnostics, U.S.A) for chemical tests (CK total, CK-MB, Tn I, LDH, and AST) except hs-CRP was analysed on Cobas c311 analyzer.

The second part of the serum was kept at – 80 °C for estimation of Lp-PLA_2 activity level on Beckman Coulter AU 400 analyzer.

Another three milliliters venous blood sample were drawn from every patient after twelve hours fasting and emptied in a plain tube then the serum was analyzed on Dimension RxL Max analyzer. (Siemens Healthcare Diagnostics, U.S.A) for fasting serum glucose and lipid profile (TG, LDL, HDL, total cholesterol).

In the present study, we found no statistically significant difference between the two studied groups regarding gender, age and smoking.

On the other hand we detected a statistically significant difference between patients and controls regarding hypertension.

We also performed lipid profile and serum glucose as a risk factors; we detected that triglycerides, total cholesterol, LDL-C, HDL-C and serum glucose were higher in patients than controls with a statistically significant difference between the two studied groups.

In the present study we estimated multiple cardiac biomarkers including CK, CK-MB mass, troponin, AST, LDH and hs-CRP. We found a statistically significant increase in patients when compared to healthy controls.

In our study, we found that Lp-PLA_2 activity level was higher among patients compared to healthy controls and was associated with increased incidence of myocardial infarction.

We detected no statistically significant difference between Lp-PLA_2 activity and gender, smoking or hypertension.

In the present study, we estimated the correlation between Lp-PLA_2 activity and different parameters. We found significant positive correlation of Lp-PLA_2 activity with triglycerides and LDL-C. We also found no correlation of age, total cholesterol, CK, CK-MB mass, troponin, LDH, AST, hs-CRP or glucose with Lp-PLA_2 activity among cases. Also there was an inverse correlation between Lp-PLA_2 activity and HDL-C in cases. No correlations were observed between Lp-PLA_2 activity and any of the previous parameters among control group.

CONCLUSIONS

It is concluded that:

a. Serum Lp-PLA_2 activity level plays an important role in the prediction of myocardial infarction. So it may be used as a risk marker for myocardial infarction development.

b. Serum Lp-PLA_2 activity is correlated with other risk factors such as lipid profile (TG, LDL-C, HDL-C).