

# Lipoprotein-associated phospholipase A2 in relation to lipid markers: A pan-India correlation study



Sandhya Iyer, Rajkumar Kushawaha, Prachi Sinkar, Sivakumar Selvaraj, Amruta Velumani

Biochemistry Division, Thyrocare Technologies Limited, Plot No.D37/1, TTC Industrial area, MIDC, Turbhe, Navi Mumbai - 400703, India

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## ABSTRACT

**Background:** Conventional serum markers are routinely recommended to assess heart health. However, many new biochemical markers like lipoprotein-associated phospholipase A2 (Lp-PLA2) has been studied as an efficient cardiac risk marker. **Aims and Objective:** The current study was designed to link the correlation between traditional lipid markers and Lp-PLA2 in a pan-India cohort. **Materials and Methods:** The study cohort included a total of 10347 pan-Indians including 5945 males and 4402 females respectively. The serum lipid parameters estimated include triglycerides, LDL- and HDL-cholesterol as well as total cholesterol by the technology of photometry. **Results:** The frequency of high Lp-PLA2 detected in our study was 6.4% and the frequency between males at 9.3% and females at 2.5% was detected to be significant at  $p < 0.0001$ . Linear regression analysis detected significant positive association of high Lp-PLA2 with LDL-cholesterol and total cholesterol at  $p < 0.05$ , while the trend with HDL-cholesterol was also significant. However, Lp-PLA2 as an independent marker was not detected to be significant in relation to the lipid indices studied. **Conclusion:** Lp-PLA2 has been associated with risk factor for acute coronary crisis; its clinical value remains controversial. Our report though detected significant association of Lp-PLA2 with various lipid parameters, independent value remains questionable.

**Key words :** Lp-PLA2; Cardiac risk; Cholesterol; India; LDL; Triglycerides

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## INTRODUCTION

Lipoprotein-associated phospholipase A2 (Lp-PLA2) is an enzyme associated with low-density lipoproteins (LDL) and is involved in the hydrolysis of phospholipids. Studies have suggested Lp-PLA2 to have a strong pro-inflammatory role and thus is a key contributing factor for atherosclerosis, plaque stability as well as severity of coronary artery stenosis.<sup>1,2</sup> The clinical utility of Lp-PLA2 continues to be controversial as it has been proven to play a role in progression of atherosclerosis on one line while clinical trials involving Lp-PLA2 inhibitors have not shown significant favorable outcomes.<sup>3,4</sup> Lp-PLA2 has been widely proposed in the clinical context to be used for risk stratification.<sup>1</sup> Lp-PLA2 is produced by the macrophages

as well as lymphocytes which bind to LDL and oxidizes the same to produce free fatty acids and lyolecithin.<sup>5</sup>

Many cardiac markers are utilized for routine screening like cardiac troponin specifically cardiac troponin I (cTn I) for acute myocardial infarction. However, the sensitivity has been proven to be low because of its association with many other conditions like renal insufficiency, myocarditis, heart failure, etc.<sup>6</sup> In contrast, Lp-PLA2 comparison studies with traditional inflammatory and myocardial injury markers detected the former to bear higher diagnostic value pertaining to early diagnosis.<sup>2</sup> The evidence of positive association has been proven in many ethnic groups with acute myocardial infarction, cardiovascular disease (CVD) as well as incidence of coronary heart disease.<sup>7,8</sup> LDLs are

### Address for Correspondence:

Dr. Sandhya Iyer, Thyrocare Technologies Limited, Plot No. D37/1, TTC Industrial area, MIDC, Turbhe, Navi Mumbai - 400703, India.

**Phone:** +91-9820423640. **E-mail:** sandhya.iyer@thyrocare.com

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the primary target of cholesterol-lowering therapy though many studies have highlighted its atherogenic predictive strength to be good only on a population basis and weak when it comes to individual cases.<sup>1</sup> Triglycerides (TG) and their role in cardiovascular risk continue to be controversial as studies have shown elevated levels of the same to also be due to metabolic syndrome or insulin resistance.<sup>9</sup> Though the biochemical structure of TG limits its ability to directly cause any atherosclerotic CVD, a mild-to-moderate raise in levels have been shown to bear the potential to trigger the inflammation cascade which can promote atherosclerosis.<sup>10</sup> Total cholesterol (TC) has been positively associated as a strong risk factor for coronary heart disease with the effect being slightly strong for men as compared to women.<sup>11</sup> Studies have also shown a strong inverse correlation to exist between levels of high-density lipoprotein (HDL) cholesterol and coronary disease, with outcomes of Framingham study highlighting increase in HDL-C by 10mg/L to cause risk reduction of up to 2 – 3%.<sup>12</sup>

Lp-PLA2 has been reported to be a good predictor of cardiac disease by many studies. A review study based on outcomes reported by 14 observational studies involving over 20,000 participants detected the predictive ability of Lp-PLA2 at combined odds of 1.60 (95% CI, 1.36 to 1.89) towards risk of future adverse cardiac events.<sup>13</sup> Association studies have also reported on correlation of Lp-PLA2 with different parameters of CVD. One such study which evaluated the association between Lp-PLA2 and the protective effect of HDL-C on 3888 CVD adults detected the relation between HDL-C levels and risk of incident coronary heart disease to be attenuated among patients in the highest tertile of Lp-PLA2. However, this study did report on absence of consistent association between high Lp-PLA2 and risk across different HDL-C categories.<sup>14</sup> Another Nature scientific report which studied Lp-PLA2 and oxidized-LDL among young Chinese patients with acute coronary syndrome detected significant correlation between Lp-PLA2 levels and smoking, TC, LDL-C as well as oxidized LDL levels at significance  $p < 0.05$ . This study concluded Lp-PLA2 to be a marker of oxidative stress and inflammation than an independent risk factor for acute coronary syndrome among young Chinese.<sup>15</sup>

Based on this background, our study was aimed at evaluating the association and trend between Lp-PLA2 and traditional lipid parameters like triglycerides, LDL

and HDL cholesterol as well as total cholesterol in a pan-India cohort.

## MATERIALS AND METHODS

### Cohort

This retrospective analysis included data from 10,347 inclusive of 5945 males and 4402 females respectively. The need for specific informed consent was deviated from, for this retrospective analysis involved use of no patient identifiers for analysis or drawing conclusion apart from gender and testing was done in our clinical reference laboratory and not hospital-based. The entire cohort characteristics have been highlighted in Table 1.

### Estimation of Lp-PLA2 and lipid parameters

Serum Lp-PLA2 estimation was done using the Diazyme Lp-PLA2 enzymatic assay in photometry technology (Olympus AU400, Beckman Olympus). The test involved hydrolysis of the substrate 1-myristoyl-2-(4-nitrophenylsuccinyl) phosphatidylcholine at the sn-2 position producing 4-nitrophenol, a colored product measured spectrophotometrically at 405nm. The activity was calculated from the rate of change in absorbance. The critical call values were set at 275 U/L wherein any value below this was reported to be low risk and above as high risk.

Estimation for lipid parameters like TG, LDL-C, HDL-C and TC was done using dedicated enzymatic assay kits from Sekure Chemistry (Sekisui Diagnostics, UK) and estimated on photometry technology (Olympus AU2700, Beckman Olympus) as per manufacturer instructions. The quality control measures involved use of two-level serum controls from BioRad Laboratories (Hercules, CA). Trend analysis was done using LJ plots and compliance with Westgard rule was evaluated. The reference ranges for reporting were as follows; TG – 25 – 200 mg/dL; LDL-C – 85 – 130 mg/dL; Cholesterol – 125 – 200 mg/dL; HDL-C – 35 – 80 mg/dL.

## RESULTS

### Cohort analysis

Our study analysis included data from a total of 10347 individuals including 5945 males (46 +/- 14 years) and 4402 females (46 +/- 14 years) studied to assess relation and

**Table 1: Study cohort characteristics**

Cohort	N	Mean age (Years)	Mean TG (mg/dL)	Mean LDL-C (mg/dL)	Mean total cholesterol (mg/dL)	Mean Lp-PLA2 (U/L)	Mean HDL-C (mg/dL)
Males	5945	46±14	157±95	116±34	185±42	200±57	43±10
Females	4402	46±14	125±74	114±32	184±39	167±48	50±11
Total	10347	46±14	143±88	115±33	184±41	186±56	46±11

trend between Lp-PLA2 and lipid parameters like TG, TC, LDL-C and HDL-C. The average levels of each of the lipid parameter studied has been highlighted already in Table 1.

Trend analysis between Lp-PLA2 and individual lipid parameters highlighted a positive correlation. Multiple regression analysis highlighted significance of Lp-PLA2 in relation to all lipid parameters at  $p < 0.01$ . The same has been highlighted in Figure 1(a), 1(b), 1(c), 1(d).

Further analysis was also done to detect if a trend exists between normal levels of lipid parameters in relation to high Lp-PLA2 levels. No significant trend was observed when the probability of this association was put to test. The detailed analysis and outcome has been highlighted in Table 2.

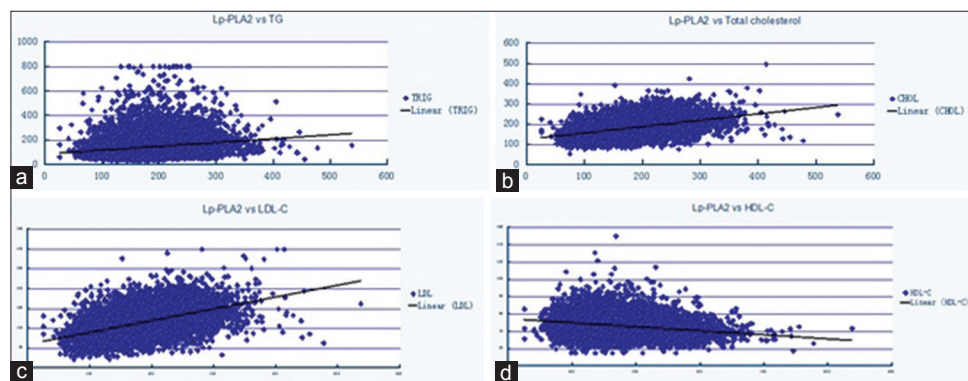
The difference in frequency between males and females in all the cases was significant at  $p < 0.0001$  by Chi-Square with Yates correction.

### High Lp-PLA2 analysis outcome

Analysis of high Lp-PLA2 values in relation to different lipid indices was also studied and highlighted in Figure 2.

Summary of frequency as well as average serum levels have been detailed in Table 3. The difference in frequency between males and females was found to be significant at  $p < 0.0001$  by Chi-Square test with Yates correction.

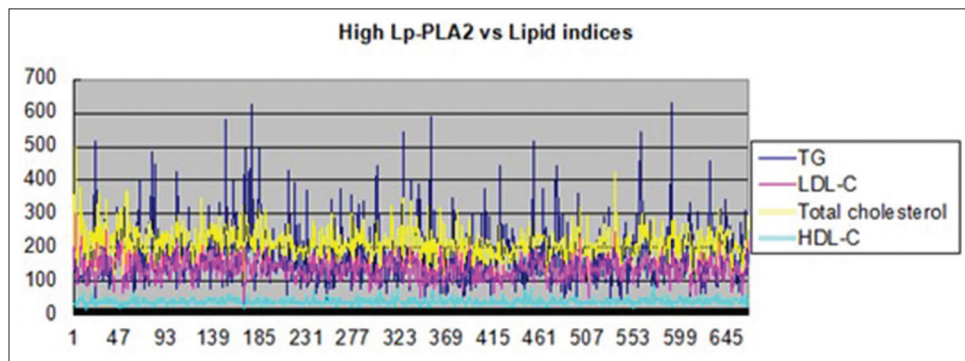
Linear regression analysis was also done to detect significance of association between each lipid parameter with high Lp-PLA2 levels. Significance was detected for LDL-C  $F(1,661) = 13.9$ ,  $p < 0.05$ , for TC  $F(1,661) = 12.1$ ,  $p < 0.05$  and for HDL-C at  $F(1,661) = 4.6$ ,  $p < 0.05$ . No significance was detected for TG with high Lp-PLA2. However when considering the strength of high Lp-PLA2 as an independent risk factor, the odds were detected to be 0.5 (95%CI, 0.4 – 0.6) for TG, 0.3 (95%CI, 0.3 – 0.4) for LDL-C and 0.3 (95%CI, 0.2 – 0.4) for TC. Further, the frequency of individuals with high values for Lp-PLA2



**Figure 1:** (a) Trend of Lp-PLA2 vs TG (b) Trend of Lp-PLA2 vs Total cholesterol (c) Trend of Lp-PLA2 vs LDL-C (d) Trend of Lp-PLA2 vs HDL-C

**Table 2: Frequency analysis outcome of normal values of lipid indices vs. high Lp-PLA2**

Cohort	Normal TG (N)	High Lp-PLA2 (N)	Frequency of high Lp-PLA2 (%)	Mean TRIG levels (mg/dL)	Mean Lp-PLA2 levels (U/L)
Males	4645	381	8.2	119±40	196±56
Females	3900	82	2.1	105±39	165±47
Total	8545	463	5.4	113±40	182±55
Cohort	Normal LDL-C (N)	High Lp-PLA2 (N)	Frequency of high Lp-PLA2 (%)	Mean LDL-C levels (mg/dL)	Mean Lp-PLA2 levels (U/L)
Males	2848	184	6.5	109±13	197±49
Females	2313	39	1.7	108±13	164±43
Total	5161	223	4.3	109±13	182±49
Cohort	Normal total cholesterol (N)	High Lp-PLA2 (N)	Frequency of high Lp-PLA2 (%)	Mean total cholesterol levels (mg/dL)	Mean Lp-PLA2 levels (U/L)
Males	3461	203	5.9	168±21	191±52
Females	2772	38	1.4	167±20	158±44
Total	6233	241	3.9	167±20	176±52
Cohort	Normal HDL-C (N)	High Lp-PLA2 (N)	Frequency of high Lp-PLA2 (%)	Mean HDL-C levels (mg/dL)	Mean Lp-PLA2 levels (U/L)
Males	4742	395	8.3	46±8	199±55
Females	4050	88	2.2	52±10	166±47
Total	8792	483	5.5	48±9	184±54



**Figure 2:** Analysis of high Lp-PLA2 in relation to estimated lipid indices

**Table 3: High Lp-PLA2 (>275 U/L) analysis outcome**

Cohort	N	Frequency (%)	Mean Lp-PLA2 (U/L)	Mean TG (mg/dL)	Mean LDL-C (mg/dL)	Mean total cholesterol (mg/dL)	Mean HDL-C (mg/dL)
Males	555	9.3	303±30	182±95	142±35	214±45	40±9
Females	108	2.5	308±35	168±74	141±37	213±50	42±9
Total	663	6.4	304±31	179±92	142±35	214±46	40±9

and all the assessed lipid parameters like TG, LDL-C and TC was detected to be 1.1% inclusive of males at 1.6% and females at 0.4% and the difference was statistically significant at  $p < 0.0001$  by Chi-Square test with Yates correction.

## DISCUSSION

Our study report aimed at determining the correlation between Lp-PLA2 and lipid parameters to identify the efficacy of the same as a good cardiac risk marker. Though the association of Lp-PLA2 as a strong risk predictor is still controversial, our aim was to identify trend association between Lp-PLA2 and conventional lipid markers.

Lp-PLA2 is secreted by the inflammatory cells in the human body and the majority ~80% is bound to lipoproteins like LDL and to a lesser extent to HDL at roughly 10%. Many studies have identified elevated Lp-PLA2 activity to be associated with risk of CVD.<sup>16</sup> Numerous studies have analyzed the strength of Lp-PLA2 as an independent risk marker in comparison with traditional cardiac risk factors and one such study which analyzed a total of 20,549 patients detected the combined odds to be 1.60 (95% CI, 1.36 – 1.89) towards development of future cardiac effects.<sup>13</sup> Another meta-analysis which reported on evaluation of Lp-PLA2 levels with stroke, CAD and mortality from a total of 79,036 participants detected significance with all three factors at relative risk of 1.08 (95% CI, 0.97 – 1.20) for stroke, 1.10 (95% CI, 1.04 – 1.17) for CAD and 1.16 (95% CI, 1.09 – 1.24) for vascular mortality.<sup>17</sup> However, our study which analyzed Lp-PLA2 specifically in relation to lipid parameters detected no such

association when frequency of high Lp-PLA2 levels was compared with normal levels of lipid indices as well as with high levels of lipids. Thus, Lp-PLA2 in comparison to traditional lipid parameters like TG, TC and LDL-C was not detected to have a significant impact towards cardiac risk independently.

A study by the Atherosclerosis Risk in Communities (ARIC) examined the relation between two inflammatory markers; Lp-PLA2 and high-sensitivity C-reactive protein (CRP) in a case-control manner. This study detected high levels of Lp-PLA2 in incident cases of coronary heart disease and also among individuals with non-elevated levels of LDL. Lp-PLA2 was detected to be independently associated with risk post adjustment for CRP and traditional risk factors.<sup>18</sup> In our study, the detected frequency of high Lp-PLA2 was 4.3% in the cohort with non-elevated LDL levels and males were found to be higher at 6.5% in comparison to females at 1.7%, significant at  $p < 0.0001$ . Though linear regression analysis detected significance of high Lp-PLA2 in relation to LDL levels, the strength of Lp-PLA2 independently was detected to be weak. One probability could be that our analysis did not take into account other traditional cardiac risk factors into consideration like BMI, blood pressure, smoking status, metabolic syndrome, etc.

A recent study among patients with acute coronary syndrome from China included 228 young patients matched with 237 control subjects and found significant correlation between Lp-PLA2 levels and smoking status, TC, LDL as well as oxidized LDL at  $p < 0.05$ . Further multivariate logistic regression detected oxidized LDL, CRP, TG, smoking and male gender to be independently associated with risk of acute coronary syndrome. However,

analysis post adjustment for oxidized-LDL, detected the association between Lp-PLA2 and acute coronary syndrome to become insignificant, indicating the risk to be dependent on levels of the later. Lack of improvement in risk prediction detected Lp-PLA2 to be a better marker of oxidative stress than CVD.<sup>15</sup> Our study detected high levels of Lp-PLA2 among individuals with normal levels of lipids, thus highlighting the risk of oxidative stress to be present irrespective of lipid levels. In our study the frequency of high lipid markers with high Lp-PLA2 levels were detected to be 1.1% with the frequency among males to be higher at 1.6% compared to females at 0.4% and significant at  $p < 0.0001$ .

Many Indian reports have also studied the efficacy of Lp-PLA2 as an emerging cardiovascular marker. One such cross-sectional study evaluated a total of 391 South Indian subjects including 271 test subjects and 120 controls. This study detected test subjects to have significantly high levels of TG, LDL, TC, random blood sugar and BP in comparison to controls at  $p < 0.001$ . The mean Lp-PLA2 levels were also detected to be high at 565.50 g/L in the test group in comparison to controls at 395.07 g/L.<sup>19</sup> In our study, subjects with high Lp-PLA2 were detected to harbor borderline levels of high TG at 179 +/- 92 mg/dL, high LDL-C at 142 +/- 35 mg/dL and TC at 214 +/- 46 mg/dL respectively. Significance with LDL-C and TC was detected by linear regression analysis at  $p < 0.05$ . Another report studied association between Lp-PLA2, oxidized LDL and glycaemic status in early stages of type 2 diabetes including 40 individuals each of case and control. This study detected significantly higher levels of the same in cases as compared to controls and a positive correlation with oxidized LDL among cases ( $r = 0.542$ ,  $P < 0.01$ ) and controls ( $r = 0.414$ ,  $P < 0.01$ ), thus proving activity of Lp-PLA2 to be highly affected by oxidized LDL.<sup>8</sup> Another Indian report which studied Lp-PLA2 and carotid intima media thickness (CIMT) as markers of increased CVD risk among 70 subjects each in case and controls, detected no relationship between predicting CIMT and Lp-PLA2 activity.<sup>20</sup>

## CONCLUSION

The significance of Lp-PLA2 as an independent cardiac risk marker continues to be studied in comparison to many biochemical markers. Highlighting a single association with proven end-point of improved patient outcome needs more clinical trial studies in large population size. Patient management using Lp-PLA2 levels and its monitoring is not currently a recommendation. Though the background for evidence remains controversial, our study does show a positive trend between high levels of Lp-PLA2 with total

cholesterol, LDL-C as well as HDL-C and hence these can be monitored together for patient management.

## ETHICAL APPROVAL

Not required. This study involved data analysis from a reference laboratory and only details pertaining to gender and age was taken for analysis. No other patient identifiers were noted or mentioned in the manuscript to the extent of compromising patient confidentiality.

## ACKNOWLEDGEMENT

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#### Authors Contribution:

**SI-** Concept and Study design, Analysis and Interpretation of data and manuscript preparation; **RK-** Data acquisition; **PS-** Concept and study design, Analysis and interpretation of data, Revision of manuscript; **SS-** Concept and study design, drafting of manuscript; **AV-** Data acquisition, revision of manuscript.

#### Work attributed to:

Department of Biochemistry, Thyrocare Technologies Limited

#### Orcid ID:

Dr. Sandhya Iyer - <https://orcid.org/0000-0002-8872-0322>  
Mr. Rajkumar Kushawaha - <https://orcid.org/0000-0001-5646-2229>  
Dr. Prachi Sinkar - <https://orcid.org/0000-0002-3336-5140>  
Dr. Sivakumar Selvaraj - <https://orcid.org/0000-0002-6912-1906>  
Ms. Amruta Velumani - <https://orcid.org/0000-0002-8337-247X>

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