CHAPTER 4
Lipoprotein-a and CAD

4.1. Introduction

Lipoprotein (a) is an antigen identified by Kare Berg in 1963 and was found to have an autosomal dominant mode of inheritance. Lp(a) is a macromolecular complex found in human plasma that combines with the structural elements from lipoprotein and blood clotting systems. It is related to LDL-C in its structure (Figures 22 and 23) and its function; has a higher density range compared to LDL-C. Due to the structural similarity with LDL-C it is considered to be proatherogenic. However Lp(a) differs from LDL-C by the presence of a highly glycosylated protein apolipoprotein (a), a dimer of Apo B -100 found in LDL-C sharing homology with plasminogen. It acts as a competitive inhibitor of tissue type plasminogen activator and modulates the fibrinolytic system by antagonistically binding to plasminogen binding sites. Therefore Lp(a) is also prothrombotic or antifibrinolytic (Braunwald, Zipes, Libby and Bonow, 2005). Lp(a) levels are known to exhibit significant inter-individual variation and are associated with an increased risk of CAD and renal failure in hypertensive patients, but only if LDL-C levels are also high (Dahlen and Stenlund, 1997; Fytili, et al., 2002). As Lp (a) levels are genetically determined, earlier screening for Lp (a) levels in asymptomatic individuals has been suggested to identify the subjects at risk.
4.2. Background and Objectives of the study

The main objectives of the study were to evaluate the effect of serum Lp(a) levels, CLTI (Comprehensive Lipid Tetrad Index) and to study the potential of Lp(a) as a more reliable marker for CAD compared to other lipids and lipoproteins. 125 CAD patients and 125 controls were recruited for the study. Their serum plasma Lp(a) levels and complete lipid profile were assayed. Serum concentrations of Lp(a) were estimated using Lp(a) latex DAIICH by immuno-turbidimetric method. Lipoprotein profile has been investigated widely in recent years, which is
found to be deranged in large proportion of CAD patients. However, a significant proportion of patients have a normal lipoprotein profile (Vasisht, et al., 1990). There is a need to study such patients in greater detail and identify some other parameters, which may indicate their CAD risk.

The lipid abnormality associated with type 2 diabetes typically consists of elevated triglycerides, LDL-C and decreased HDL-C level. The frequently mild abnormality in LDL-C concentration associated with diabetes belies a qualitative abnormality in the LDL-C structure, i.e., decreased size and increased density of the LDL-C particle (Mohan, Venkatraman and Pradeepa, 2010). Even when LDL-C is normal or within a range that might be considered low in diabetic individuals, LDL-C appears to be very potent contributor to the development of CAD (Howard, et al., 2000). In addition to VLDL-C, LDL-C levels are also somewhat increased in diabetic individuals under poor control, probably accounting in part for their increased risk for CAD. Lp (a) is also accepted an atherogenic lipoprotein when its plasma level is above 25 mg/dl; however levels can vary in different ethnic groups (Obisesan, et al., 2004). There are some studies that evaluated the relationship between Lp(a) and glycemic control in diabetic patients (Haffner, Tuttle and Rainwater 1991, 1992b, Haffner, Morales and Stern,1992a and Haffner, Moss, Klein and Klein, 1992c), but with inconclusive results regarding its association with type 2 diabetes (K.C.Khare, et al., 2000), and not much explored in South Indian population.
Moreover little is known about the influence of lipid profiles on serum Lp(a) concentrations. Some authors have found a positive correlation between Lp(a) serum levels and LDL-C in non-diabetic patients (Bovet et al. 1994; Contois et al. 1996, Goel, et al., 2003). However, this correlation might be partly explained.

In addition, although an inverse correlation between Lp(a) and triglycerides has been reported in non-diabetic subjects, there are no specific studies on this issue in the diabetic population. It has been demonstrated that diabetic patients have a high risk of CAD (Miettinen, et al., 1998: Haffner, et al., 1998). Thus, a correlation study of the relationships between Lp(a) and other lipid measures in these patients is crucial.

Moreover, Comprehensive Lipid tetrad index (CLTI) derived by the product of cholesterol, triglycerides and Lp(a) values divided by the HDL-C level may be the best estimate of the total burden of dyslipidaemia as it eliminates the need for various cut-off points and ratios involving the lipid subsets. A high index (>20,000) would indicate the presence of a highly atherogenic lipid profile. This index can serve as a better and novel risk factor for CAD and has been determined in few studies involving South Indian population (Rajappa, Shridhar, Balachander and Sethuraman, 2006). Therefore the present study was planned to evaluate the relative sensitivity and specificity of the lipid subsets as risk factors in the prediction of coronary events and to evaluate Lp(a) and CLTI as potential predictors of CAD.
4.3. Literature Review

4.3.1. Role of Lp(a) in CAD

In 1979, Berg et al. reported an association between Lp(a) and CAD. Atherogenic as well as anti-fibrinolytic properties of the Lp(a) particles may be of pathogenic importance (Berg, et al., 1979). Lp(a) levels correlate with both early and advanced atherosclerosis, with respect to severity, extent, progression and complications of CAD including re-stenosis following percutaneous transluminal angioplasty, stent and bypass surgery.

Table 9: Association of level of Lp(a) and levels of other lipoproteins to CAD risk in Global and Indian Population

<table>
<thead>
<tr>
<th>Author</th>
<th>Sample size</th>
<th>Lp(a)</th>
<th>Other Lipids and Lipoprotein Levels</th>
<th>CAD Risk</th>
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<tr>
<td><strong>Global Population</strong></td>
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<td>Ronald, et al., (2011)</td>
<td>530</td>
<td>↑</td>
<td>Lp-PLA2↑</td>
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<tr>
<td>Kardiologiia, (2011)</td>
<td>361</td>
<td>↑</td>
<td>-</td>
<td>↑</td>
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<tr>
<td>Massot, et al., (2011)</td>
<td>75</td>
<td>↑</td>
<td>-</td>
<td>↑</td>
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<tr>
<td>Song, et al., (2011)</td>
<td>14</td>
<td>↑</td>
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<td>↑</td>
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<tr>
<td>Barra, et al., (2011)</td>
<td>77</td>
<td>↑</td>
<td>HDL-C↑, TC↑</td>
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<td>↑</td>
<td>-</td>
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<td>145</td>
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<td>El Oudi, et al., (2011)</td>
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<td>LDL-C↑</td>
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</table>
Lipoprotein-a and CAD

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Lp(a) excess, increases the risk of premature CAD 3 to 100 fold depending on the absence or presence of concomitant risk factors (Hopkins, et al., 1997). The risk of Lp(a) lies in the truth that it is 10 times more atherogenic than LDL-C. Moreover steady life-long levels of Lp(a) is attained right from infancy; therefore pathological processes associated with elevated Lp(a) level also set in motion in infancy, about 20 years earlier in contrast to other risk factors for CAD like hypertension, cigarette smoking and other dyslipidaemias (Enas and Yusuf 1999).

The gene coding for Lp(a) is inherited on responsible chromosome 6. It is heritable in a Mendelian dominant manner which means around 50% of offsprings of...
parents with elevated Lp(a) will also have higher Lp(a) (Superko and Krauss, 1996). There exist more than 25 inherited forms of Lp(a), resulting in a large difference of plasma levels of this lipoprotein across diverse ethnic inhabitants (Braunwald, Zipes, Libby and Bonow, 2005). Structurally LDL-C particles have 1 mole of apolipoproteins B-100 (Apo B-100) per particle. In Lp(a) Apo B-100 is connected by a disulfide bond to apo(a). Compared to Apo B-100, which is relatively constant in weight, the weight of apo (a) moiety ranges in between 300 and 800 kilo Daltons (Scanu, 2003). There exists a robust correlation between phenotypic forms of Lp(a) and their plasma levels and probably also atherogenecity. The larger isoforms (S3, S4 and >10 PN repeats) of apo (a) are believed to be less atherogenic when compared to smaller (S1, S2 and 6-9 PN repeats) isoforms. The smaller isoform is linked with higher plasma Lp(a) levels whereas larger isoforms with lower Lp(a) levels (Vasisht, et al., 2000; Geethanjali, Jose Jacob and Kanagasabhapathy, 2002).

There is a close structural homology between Lp(a) and plasminogen. This has raised the possibility that this lipoprotein competes with plasminogen and prevents it’s binding to the vascular endothelium thus inhibiting fibrinolysis (Braunwald, Zipes, Libby and Bonow, 2005).

Lp(a) levels are affected by a wide variety of factors including age, sex and ethnic background. Ethnicity has a significant association with Lp(a) level (Obisesan, et al., 2004). Normal plasma Lp(a) concentration reported for Western population is less than 20 mg/dl (Berg, 1991). In a comparative study on different
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ethnic groups, South Asians settled in North America were reported to have high Lp(a) levels compared to Caucasians, Chinese and Malays (Anand, et al., 1998), (Table 9). The mean values given for Lp(a) in a Framingham cohort are 14 mg/dL for men and 15 mg/dL for women. The majority of the studies on Lp(a) have been executed on Whites and data on other ethnic groups are scarce. In Asian Indians, raised Lp(a) levels are a powerful risk factor for premature CAD, which occurs 3-10 times more in young Asians less than 40 years of age as compared to other populations (Enas, 1996). The threshold for therapeutic decisions for serum Lp(a) is considered to be >30 mg/dl; strangely values exceeding this threshold are found to be more prevalent in Asian Indians when compared to several other ethnic groups (Enas, Dhawan and Petkar, 1997). Indian population is going through epidemic transition as a consequence of affluence, urbanization and mechanization.

Further, significant relationship of Lp(a) with CAD is reported in few South Indian studies (Mohan Deepa and Rema, 1998; Kanagasabapathy, 2002). Study of Lp(a) will facilitate the process of identifying the risk factors associated with the malignant nature of CAD in Indian population. Also quantifying Lp(a) levels in diverse populations can help in identifying the high risk group requiring aggressive pharmacological management (Wang, Cranney and Wilcken, 2000).

4.3.2. Lp(a) in CAD and diabetes mellitus

The contribution of Lp(a) to the increased risk of atherosclerosis in subjects with diabetes has not been established clearly. The data on relationship between Lp(a) and diabetes is scarce and the data on Lp(a) in Asian Indian diabetics is still meager and controversial. Some reports found that Lp(a) is an independent risk
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factor for CAD in diabetics (Velho, et al., 1993; Watts, et al., 1995) while others were unable to show a significant relationship between Lp(a) and CAD (Haffner, et al., 1992c; Niskanen, Mykkanen, Karonen and Uusitupa, 1993; O’Brien, et al., 1994).

CLTI proposed by Enas EA (Enas, 2000), was designed to magnify the subtle abnormalities of the various atherogenic and anti-atherogenic lipoproteins (Gupta, et al., 2000) and is derived by multiplying the three commonly measured lipids directly associated with CAD and dividing the product by HDL-C, which is inversely associated with CAD \((\text{total cholesterol} \times \text{triglyceride} \times \text{Lp(a)} / \text{HDL-C})\).

In addition, although an inverse correlation between Lp(a) and triglycerides has been reported in non-diabetic subjects, there are no specific studies on this issue in the diabetic population. It has been demonstrated that diabetic patients have a high risk of CAD (Miettinen, et al., 1998; Geethanjali, Jose Jacob and Kanagasabhapathy, 2002). Thus, an accurate study of the relationships between Lp(a) and other lipid measures in these patients could be particularly revealing. The current study is undertaken to address the role of Lp(a) and CLTI as determinants of CAD with and without DM.

4.4. Materials and Methods

4.4.1. Estimation of Serum Lp(a)

Serum concentrations of Lp(a) were estimated using Lp(a) latex DAIICHI kit (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) by immuno-turbidimetric method, using semi auto analyser (Star 21 plus, Rapid Diagnostics, USA).
Kit Contents:

- Buffer solution: glycine buffer (50 mmol/L, pH 9.0)
- Latex reagent: anti human Lp(a) mouse monoclonal antibody coated latex (1.6 mg/mL).

Method:

Lp(a) in serum or plasma reacts with antihuman Lp(a) mouse monoclonal antibody coated latex and agglutination occurs. Lp(a) concentration is determined by measurement of the change in turbidity that results from the agglutination reaction of Lp(a) with the antibody coated latex. The reaction sequences of the assay are shown below:

Lp(a) + Antihuman Lp(a) mouse monoclonal antibody coated latex → Immune complexes → absorbance measurement

Preparation:
Reagent 1: Buffer solution is a ready-to-use reagent.
Reagent 2: Latex reagent is a ready-to-use reagent.

Procedure:

\[
\begin{align*}
\text{Sample} & \rightarrow \text{Reagent (1)} & 37^\circ \text{C} & \rightarrow \text{Reagent (2)} & 37^\circ \text{C} & \rightarrow \text{Measurement (absorbance I*)} \\
(3 \mu\text{L}) & \rightarrow (300 \mu\text{L}) & 5 \text{ min} & \rightarrow (100 \mu\text{L}) & 1 \text{ min} & \\
37^\circ \text{C} & \rightarrow \text{Measurement (absorbance II*)} & \rightarrow \text{Calculation of Lp(a) concentration} \\
4 \text{ min} & & & & \\
\end{align*}
\]

Sensitivity:

1. Reagent blank: Less than 0.02 Abs.
2. Sensitivity: 0.04-0.20 Abs. per 20.0 mg/dL Lp(a)

Specificity:

90-110% of assay expected values

Precision:

Coefficient of variation is less than 5% (within-run)

4.4.2. Statistical Analysis

Descriptive statistics were used to summarise the clinical findings, risk factors, and coronary angiographic findings of patients. Student’s t test was used to get the statistical significance. Pearson’s correlation co-efficient was used to find the association between Lp(a) and other cardiovascular risk factors. The association between individual risk factor and outcome was estimated using univariate logistic regression. The multivariate logistic regression analysis was used to estimate the effect of Lp(a) on CAD, controlling the other confounders. A value of P<0.05 was taken as significant.

4.5. Results

The subjects with CAD had higher Lp(a) levels (Mean ± SEM=40.88 ± 0.994mg/dl; P<0.001) than that of the control group (Mean ± SEM=28.96 ± 0.4mg/dl) (Figure 24). Also the subjects with CAD had higher CLTI levels (Mean ± SEM=35538.55 ± 2370.58 (mg/dl)²; P<0.01) when compared to subjects without CAD (Mean ± SEM=20779.78±1442.908 (mg/dl)²) (Figure 25).
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Figure 24: Lp(a) levels in relation to CAD

Figure 25: CLTI levels in relation to CAD

The characteristics of the subjects who had DM with and without CAD are shown in Figure 26. Serum Lp(a) levels of patients having CAD with DM (Mean ± SEM =42.75 ±1.3053 mg/dl) and CAD patients without DM (Mean ± SEM =38.24±1.4799 mg/dl) were significantly higher (P<0.05) than the diabetic patients without CAD (Mean ± SEM= 30.09± 0.640 mg/dl) and patients without CAD and DM (Mean ± SEM=27.78± 0.603 mg/dl) respectively (Figure 26). Also CLTI levels of CAD patients with DM (Mean ± SEM=39426.6±3508.498 (mg/dl)^2) and CAD patients without DM (Mean ± SEM=30080.33±2729.132 (mg/dl)^2) were
significantly higher (P<0.05) than the diabetic patients without CAD (Mean ± SEM=22084.74±1846.174 (mg/dl)^2) and patients without CAD and DM (Mean ± SEM=19387.83± 2241.54 (mg/dl)^2) (Figure 27).

Figure 26: Lp(a) levels in relation to CAD and DM

![Graph showing Lp(a) levels in relation to CAD and DM](image)

*P < 0.05 when compared to subjects without CAD

Figure 27: CLTI levels in relation to CAD and DM

![Graph showing CLTI levels in relation to CAD and DM](image)

*P < 0.05 when compared to subjects without CAD

Further, the mean Lp(a) levels obtained for subjects diagnosed with CAD with SVD, DVD and TVD were compared in subjects without CAD (Figure 28a). A highly significant increasing trend in Lp(a) levels was observed with the rise in
angiographic severity of CAD: No CAD (Mean ± SEM = 29.04±0.456mg/dl) < SVD (Mean ± SEM= 39.86±1.92mg/dl) < DVD (Mean ± SEM= 40.26±1.82mg/dl) < TVD (Mean ± SEM= 41.64±1.54mg/dl)  (P< 0.05). Moreover, the mean Lp(a) levels obtained for subjects diagnosed with CAD in terms of extent of myocardial ischemia as computed by Gensini Scores in SVD, DVD and TVD were compared in subjects without CAD (Figure 28b). A highly significant increasing trend in Lp(a) levels was observed with the rise in severity of CAD: SVD (Mean ± SEM=12.91 ± 2.12 mg/dl) < DVD (Mean ± SEM=37.22 ± 4.04 mg/dl) < TVD (Mean ± SEM= 67.51 ± 4.96 mg/dl)  (P< 0.05). Higher mean concentrations of serum Lp(a) were seen with more advanced grades of CAD.

Figure 28a: Lp(a) levels in relation to angiographic severity of CAD
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Figure 28b: Lp(a) levels in relation to stenosis scores in SVD, DVD & TVD

Figure 29: Lp(a) levels in relation to family history of CAD

Figure 30: Lp(a) levels in relation to smoking
Higher Lp(a) levels were observed (Figure 29), in individuals with a strong family history of CAD (Mean ± SEM=38.4±1.14 mg/dl; P<0.01) than in those without such history (Mean ± SEM = 32.22±0.7 mg/dl). Compared to controls, subjects with a family history of CAD had higher total cholesterol, LDL-C, triglycerides, and Lp(a). Also higher serum Lp(a) were found to be in smokers (Mean ± SEM=39.62±1.76 mg/dl; P<0.01) when compared to non-smokers (Mean ± SEM=34.22±0.73 mg/dl) (Figure 30)

Table 10 summarises the association of Lp(a) with various cardio vascular risk factors like age, BMI, waist circumference, waist hip ratio (WHR), blood pressure, sugar and lipid profile in the study population. There was no significant association between age, BMI or waist circumference and Lp(a) levels. Blood pressure had a good correlation with Lp(a) (P<0.001). Among the sugar profile fasting blood sugar, glycated hemoglobin and HOMA IR were found to be significantly correlated with rise in Lp(a) levels (P<0.001). Among the lipid subsets, serum triglycerides and TG/HDL-C were found to be significantly associated with Lp(a) levels (P<0.001) followed by LDL-C (P<0.05).

Furthermore, in patients with CAD, predictive surrogate markers like troponin t (r = 0.378), CPK (r = 0.454), CPK-MB (r= 0.471), LDH (r = 0.366) were assessed and the results were correlating significantly with elevated Lp(a) levels (P<0.001). The extent of myocardial ischemia as assessed by Gensini score (Mean ± SEM = 0.456) also significantly correlated with Lp(a) (P<0.001).
Table 10: Pearson correlation analysis of Lp(a) with cardiovascular risk factors

<table>
<thead>
<tr>
<th>Risk Factors</th>
<th>Lp(a) (mg/dl)</th>
<th>r value</th>
<th>P value</th>
</tr>
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<tr>
<td>Age (Years)</td>
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<td>Body mass index (kg/m²)</td>
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<td>Waist circumference (cm)</td>
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<td>Waist Hip Ratio (WHR)</td>
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<td>Systolic blood pressure (mm Hg)</td>
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<td>0.188</td>
<td>0.003**</td>
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<tr>
<td>Diastolic blood pressure (mm Hg)</td>
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<td>HOMA IR</td>
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<td>Fasting Blood Sugar (mg/dl)</td>
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<td>Glycated hemoglobin (%)</td>
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<td>Serum cholesterol (mg/dl)</td>
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<td>0.023*</td>
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<td>Serum triglycerides (mg/dl)</td>
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<td>&lt; 0.001**</td>
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<td>HDL cholesterol (mg/dl)</td>
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<td>LDL cholesterol (mg/dl)</td>
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<td>Non HDL cholesterol (mg/dl)</td>
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<td>Cardiac Markers</td>
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<td>Troponin t levels (ng/dl)</td>
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<td>&lt; 0.001**</td>
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<tr>
<td>CPK (u/l)</td>
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<td>0.454</td>
<td>&lt; 0.001**</td>
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<td>CPK-MB (u/l)</td>
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<td>LDH (u/l)</td>
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<td>Stenosis score</td>
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<td>0.456</td>
<td>&lt; 0.001**</td>
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**Correlation is significant at 0.01 level

*Correlation is significant at 0.05 level
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Table 11: Multiple logistic regression analysis using CAD as dependent variable

<table>
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<tr>
<th>Parameter</th>
<th>Odds Ratio [OR]</th>
<th>95% Confidence Interval [CI]</th>
<th>P value</th>
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<td><strong>Independent variable: Lp(a)</strong></td>
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<td>Model 1: Lp(a) - Unadjusted</td>
<td>1.245</td>
<td>1.161 – 1.335</td>
<td>&lt;0.001</td>
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<tr>
<td>Model 2: [Model 1 + adjusted for age and gender]</td>
<td>1.249</td>
<td>1.157 – 1.348</td>
<td>&lt; 0.001</td>
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<tr>
<td>Model 3: [Model 2 + adjusted for insulin resistance]</td>
<td>1.243</td>
<td>1.151 – 1.343</td>
<td>&lt; 0.001</td>
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<tr>
<td>Model 4: [Model 3 + FBS]</td>
<td>1.249</td>
<td>1.153 – 1.354</td>
<td>&lt; 0.001</td>
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</tbody>
</table>

Table 11 depicts the multiple logistic regression analysis using CAD as dependent variable. Areas under the curve of Model 1 (Lp(a)-Unadjusted), Model 2 (Model 1 + adjusted for age and gender), Model 3 (Model 2 + adjusted for insulin resistance) and Model 4 (Model 3 + FBS) of regression model were 1.245 (95% CI: 1.161 – 1.335, P< 0.001), 1.249 (95% CI: 1.157 – 1.348, P< 0.001), 1.243 (95% CI: 1.151 – 1.343, P < 0.001) and 1.249 (95% CI: 1.153 -1.354, P<0.001), respectively to predict CAD. Lp(a) was thus found to be significantly associated with CAD even after adjustment with age, gender and fasting blood sugar thereby indicating the independent role of Lp(a) as CAD risk factor.

4.6. Discussion

Asian Indians worldwide have maximum rates of morbidity and mortality from CAD despite the fact that in actuality, half of them are all-time pure vegetarians. They have 2-4 fold higher risk of CAD irrespective of gender, religion, social class and develop 5-10 years earlier than in other populations;
occurrence of first MI <40 years of age is 5-10 fold higher. Surprisingly the higher rate of CAD in India is sharp in contrast to other Asian countries like China, Hong Kong, Japan and Taiwan, with reports of highest rate of smoking and hypertension. This strange trend suggest that some genetic factor is most probably contributing to escalating CAD in Indians, which is none other than Lp(a) - an emerging novel risk factor.

The median level of Lp(a) is considered to be 16 mg/dl in Asian Indians, 22 mg/dl in blacks, 6 mg/dl in whites and 3 mg/dl among American Indians respectively (Bhatnagar, et al., 1995; Anand, et al., 1998; Wang, et al., 2002). In the Indian context higher mean levels of Lp(a) were observed in cases than control in studies which ranged from 12 to 41 mg/dl in patients and 8 to 24 mg/dl in controls (Kim, and Song, 1994; Jose, et al., 1997; Mohan Deepa and Rema, 1998; Gambhir Gambhir D.S, and Morreset, 1998 and Gambhir, Haarsimrut and Gambhir D.S, 2000; Muthuswamy, Amuthan and Kumaravel, 2000; Vasisht, et al., 2000; Gupta and Gupta V.P, 1996a; Enas and Senthilkumar, 2002). In the current study the subjects with CAD had significantly higher mean Lp(a) concentrations (40.88 mg/dl; P<0.001) than the control group, which is higher than reported in the Western population explaining the higher prevalence of CAD reported in South India.

The comparatively higher mean value of Lp(a) in our study, even when adjusted for age, gender and fasting blood sugar proved Lp(a) to be an independent risk factor CAD. This finding synchronize also with various case-control studies.
done on South Asians, which showed a genetic disposition with higher Lp(a) levels than their Western counterparts (Enas, et al., 2007; Rambihar, 2002; Hoogeveen, et al., 2001; Gupta, Singh and Verma, 2006). In a recent study conducted in 2007, elevated Lp(a) levels were causally related to more severe CAD, and it was recommended that while assessing cardiovascular risk in a CAD patient, Lp(a) levels should be considered (Moon, et al., 2007). Most of the case-control studies of Lp(a) among Indians (Hoogeveen, et al., 2001; Palaniappan, et al., 2002; Tavridou, Unwin, Bhopal and Laker, 2003; Geethanjali, et al., 2003; Ranjith et al., 2004; Ashavaid, et al., 2005; Sharma, et al., 2006; Das, Daga and Gupta, 2007; Enas, et al., 2008; Goswami, et al., 2009) and global population correlate and mimic the findings of our study (Kochan, et al., 2010; Racherla and arrora, 2010; Cho, et al., 2010; Heart Protection Study Collaborative Group, 2010; Erqou, et al., 2010; Casas, et al., 2010; He, Xie, Ding and Chen, 2010; Canouï-Poitrine, et al., 2010; Boden-Albala, et al., 2010; Nordestgaard, et al., 2010; Lp-PLA(2) Studies Collaboration, et al., 2010; Vaisar, et al., 2010; Ezhov, et al., 2011; Song, et al., 2011; Berger, et al., 2011; Barra, et al., 2011; Jang, et al., 2011; El Oudi, et al., 2011; Hlebowicz, et al., 2011; Nenseter, et al., 2011; Kinney, et al., 2011; Lippi, Franchini and Targher, 2011; Kivimäki, et al., 2011).

In the present study, a distinct prototype of atherogenic dyslipidemia was observed in the study groups, characterized by high serum concentrations of TG, LDL-C and low HDL-C.
This pattern was reverberating in few Indian studies conducted by Ramachandran, et al., (2001), Goel, et al., (2003), and Tewari, et al., (2005). This symbolic pattern of dyslipidemia, along with elevated genetically determined Lp(a) concentrations, results in a “fatal atherogenic lipid tetrad” in subjects with Type 2 DM and acts as risk factor for premature CAD, necessitating earlier aggressive therapy to avert adverse outcomes, as suggested in other studies (Yeolekar, 1998; Gupta, et al., 2000; Enas, 2000).

Moreover, the effect of various lipid parameters as well as Lp(a) on the atherogenicity is additive but multiplicative which is well demonstrated by the CLTI. It reflects the subtle aberrations of the various atherogenic and anti-atherogenic lipoproteins (Rajappa, Shridhar, Balachander and Sethuraman, 2006). In the present study subjects with CAD had statistically significant higher mean CLTI level of 35538.55mg/dl when compared to the mean CLTI level of the subjects without CAD which was 20779.78 mg/dl (P<0.001). This observation is in accordance with the findings of earlier studies done by Rajappa, Shridhar, Balachander and Sethuraman, 2006 and Enas, (1993 and 1996). Likewise, a review done by Yeolekar showed that Asians had a deadly lipid tetrad index which becomes the single predictor of CAD in Asian region (Yeolekar, 1998). Total cholesterol, Lp(a) and CLTI have higher specificity (100%) and positive predictive value (100%).

Significant difference in Lp(a) levels was observed with angiographic severity and extent of CAD in the present study. This finding is in harmony with
other studies (Gupta and Gupta V.P, 1996b; Matsumoto, et al., 1998; Gazzaruso, et al., 1998; Uusmaa, Kervinen, Kesaniemi and Peukurinen, 2002; Peltier, et al., 2002).

There are conflicting data concerning Lp(a) in type 1 diabetic, type 2 diabetic and non-diabetic patients. Raised serum Lp(a) levels have been reported in type 2 DM patients in comparison with non-diabetic subjects (Ramirez, et al., 1992; Velho, et al., 1993) unlike other studies (Boulton, et al., 1985; O’Brien, et al., 1994; Haffner, Tuttle and Rainwater, 1992b; Haffner, Moss, Klein and Klein, 1992c and Haffner, 1993). The same inconsistent data also prevail in type 1 DM patients. Guillauseau, et al., (1992), Nagashima, et al., (1993) and Guerci, Meyer and Sommer, (1999) have reported elevated Lp(a) in type 1 DM while Austin, Vijay Warty., Janine Janosky, and Silva Arslanian., (1993), Gall, et al., (1992) and Calamazra and Vella (1999), found no significant increase in Lp (a) in type 1DM patients as compared to controls. Moreover certain studies have indicated that Lp(a) concentrations are elevated in subjects with Type 1 DM (IDDM), especially in those with poor metabolic control (Jenkins, Steele, Janus and Best, 1991), while improved metabolic control resulted in decrease of Lp(a) in type I DM (Haffner, Tuttle and Rainwater, 1991). In the meantime, some authors have found a positive correlation between Lp(a) serum levels and LDL-C in non-diabetic patients (Bovet, et al., 1994; Contois, et al., 1996).

A relatively few studies have been performed on Lp(a) to CAD in diabetics (Mohan V, et al., 1988; Rajappa, Shridhar, Balachander and Sethuraman, 2006).
especially in Indian population. In the present study elevated Lp(a) concentrations established a strong correlation with CAD in Type 2 DM patients of South India. CLTI levels were also higher in CAD patients with Type 2 DM [39426.6 (mg/dl)²] or without Type 2 DM [30080.33 (mg/dl)²] than non-diabetics without CAD [19387.83 (mg/dl)²](P<0.05). This finding especially with respect to Lp (a) is in concurrence with the conclusions of Mohan, Deepa and Reema, (1998a) and Rajappa, Shridhar, Balachander and Sethuraman, (2006) in South Indian patients and Misra, (1999) in North Indian patients and also with respect to CLTI levels (Rajappa, Shridhar, Balachander and Sethuraman, 2006) but these studies lacked angiographic confirmation of subjects (case and control) which accounts for one of the limitations as stated in these studies. However, our finding is in contrast with Khare, et al (2000).

Type 2 DM and insulin resistance syndrome (IRS) are predominatly found in Indians and are associated with increased CAD. IRS produces a prothrombotic state due to the stimulatory effects of insulin on smooth muscle and increases concentrations of platelet activator inhibitor -1(PAI -1).

Lp(a) shares a major prothrombic role in CAD pathogenesis by upregulating PAI-1 synthesis by endothelium which is found to be markedly elevated in IR states such as type 2 DM. All these effects may be augmented by concomitant dyslipidemic states. Recently, it has been proposed that in conditions like higher oxidative stress coupled with increased Lp(a) levels, a pro-inflammatory milieu may prevail, which contributes to the pathogenesis of CAD.
(Tsimikas, et al., 2005). In our study, this justification holds good for the correlation found between elevated Lp(a) levels and type 2 DM with and without CAD.

Because Lp(a) levels are highly heritable, it was of interest to see whether elevated levels were associated with a positive family history. In the current study, higher Lp(a) levels were reported in individuals with a strong family history of CAD than in those without such history (P<0.01) which is in agreement with other reports (Saibaba, et al., 2004; Durrington, et al., 1988). Also Lp(a) level in CAD patients with positive family history of DM and hypertension was higher than in with negative family history. Lp(a) level is an important determinant of CAD among patients with familial and non-familial hypercholesterolemia (Schaefer, et al., 1994). In the present study also higher Lp(a) levels were observed in patients with hypercholesterolemia than normo-cholesterolemia. The pathogenecity of Lp(a) is increased with high LDL-C and vice-versa (Von Eckardstein, Schulte, Cullen and Assamann, 2001). Lp(a) levels in our study were relatively higher in patients with LDL-C >130 mg/dl than patients having LDL-C <130 mg/dl (P<0.05). The patients with family history of CAD, hypercholesterolemia and with LDL-C >130 mg/dl showed higher Lp(a) levels. This finding indicates that these category of patients were more prone to have premature and severe CAD, as evidence shows that Lp(a) excess increases the premature risk of CAD depending on the absence or presence of concomitant risk factors which is in agreement with other reports (Mohan, Deepa and Rema, 1998b; Saibaba, et al., 2004). More over
in the present study, Lp(a) was significantly associated with serum triglycerides (P<0.001).

High concentrations of Lp(a) and CLTI, along with high prevalence of type 2 DM, may render Indians particularly vulnerable to malignant early atherogenic dyslipidemia relatively at a younger age. As type 2 DM is escalating in India, the above observations have profound significance in terms of total burden of CAD in India. Since high levels of Lp(a) have almost the same predictive values as family history of premature CAD, we advocate that “tracking” Lp(a) level from childhood may be a better alternative than detecting other dyslipidemia which are not wholly expressed until in middle age.

4.7. Conclusion

The results of the present study suggest that though TC, TG and LDL-C are important risk factors for CAD, Lp(a) shows a stronger association with CAD than these conventional lipids. As its levels are alleged to be genetically determined, it may prove to be a better diagnostic parameter of CAD risk at an early age. Also there was a statistically significant increase in the serum levels with an increase in the severity of CAD. These findings substantiates the need to support the introduction of routine assessment of Lp(a) levels in clinical laboratories in the monitoring of patients at risk for CAD once in all subjects, especially who present with premature CAD, familial hypercholesterolaemia, a family history of premature CAD and or recurrent CAD despite statin therapy.
In addition, the current study revealed an increase in Lp(a) levels along with a higher CLTI. Due to its higher sensitivity and positive predictive value, CLTI has emerged as a novel predictor of CAD in this South Indian subjects as compared to other conventional factors and lipid subsets. Various studies have already proven that Asians are having a different phenotype with higher values of Lp(a) than Western populations; however, whether the same phenotype exists in the whole of India has never been studied. This study can be used as a platform to do a larger study in diverse regions of this country to explore the roles of Lp (a) and CLTI as markers for premature CAD. This index in particular has a potential for emerging as a target for lipid lowering therapies instead of individual lipid subsets.

Moreover, high serum Lp(a) and CLTI levels showed strong correlation with CAD in type 2 DM patients of South India. As diabetic dyslipidemia is increasing in prevalence, the above observations have ominous magnitude in terms of total burden of CAD in India. Since Lp(a) is a non-modifiable risk factor, there is a dire need for earlier tracking and detection in younger individuals to curb the burden of cardio-diabetic morbidity and mortality.
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Lipoprotein-a and CAD


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