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Original Research Article

A study on Lipoprotein (a) levels in Atherosclerotic Peripheral Arterial Disease

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Abstract: Atherosclerotic Peripheral Arterial Disease (PAD) is a prototype of chronic systemic atherosclerosis, which is one of the major causes of morbidity and mortality in Indian population. Lipoprotein (a) is a genetically determined, cholesterol rich lipoprotein, high concentration of which has emerged as a prominent risk factor for the development and progression of atherosclerosis. This case control study undertaken in a tertiary care hospital aims to show the association and presumably a causative role of lipoprotein (a) in peripheral arterial disease in a south Indian population. In this study, serum lipid profile parameters like total cholesterol, triglyceride, LDL, HDL and lipoprotein (a) were measured in 50 patients of atherosclerotic PAD and statistically compared with 50 age and sex matched healthy individuals using student's t test and Chi square test. Mean serum lipoprotein (a) in cases was significantly higher ($p<0.01$) than that of controls. Similarly, mean serum total cholesterol, triglyceride and LDL cholesterol were significantly higher in cases than in controls ($p<0.01$), with mean serum HDL being significantly lower in cases ($p<0.01$). There was a strong negative correlation between the levels of HDL and lipoprotein (a). These results show that elevated lipoprotein (a) is an independent risk factor for the development of atherosclerotic peripheral arterial disease in the study population.
Keywords: Atherosclerosis, Peripheral Arterial Disease, Lipoprotein (a)

INTRODUCTION

Peripheral Arterial disease (PAD), or Peripheral Vascular Disease, is the atherosclerotic arterial occlusive disease affecting the lower extremities [1]. Chronic lower extremity ischemia is a common cause of loss of walking ability and is associated with a constellation of disorders affecting the entire system. Atherosclerotic PAD is a major cause of morbidity and mortality especially affecting the elderly population [2]. The prevalence is manifold higher in diabetics [3], probably because of hyperglycemia, hypertension, hyperlipidemia, platelet factors etc [4]. Conventionally PVD is diagnosed by measuring the Ankle Brachial Pressure Index (ABPI) or Ankle Brachial Index (ABI) calculated as follows:

$$\text{ABI} = \frac{\textbf{Ankle systolic Pressure}}{\textbf{Highest Brachial systolic pressure}}$$

>/= 1 : normal; 0.5 to 1 : moderate disease; < 0.5 : severe disease; <0.3 : critical limb ischemia

PAD is diagnosed when the ABI is <0.9 [6].

Dyslipidemia is a major risk factor for the development and progression of atherosclerosis, along with the life style and co morbid conditions like Diabetes mellitus. Lipoprotein(a) (Lp(a)) is a highly atherogenic lipoprotein that is under strong genetic control by the LPA gene locus [7]. It is synthesized in the liver and possibly catabolised in the kidneys; no specific receptor has been identified [7,8]. High Lp (a) concentration represents an indicator of risk for cardiovascular disease, especially when serum LDL-cholesterol or Apo B are elevated. Lp(a) levels are found to be resistant to standard lipid lowering therapy, with the exception of Niacin [9]. Lp(a) consists of an LDL-like particle and the specific apolipoprotein(a) [apo(a)], which is covalently bound to the apoB100 of the LDL like particle through disulphide bridges[7,8] (Fig.1). Each Lp[a] particle contains one molecule of apo[a], which has a number of size isoforms due to a variable number of kringle 4 (K4) repeats, so called because of their resemblance to the K4 domain of

plasminogen (Fig 2). The physiological function of Lp(a) is still unclear. The high homology of apo(a) and plasminogen suggested that Lp(a) may act as a modulator of the balance between bloodclotting and fibrinolysis. The pathogenic role of lipoprotein(a) has long been a mystery, more mechanisms of action being discovered as researchers probe into it. Researches have shown that Lp(a) promotes atherogenesis due to the LDL particle, inhibits fibrinolysis and causes athero thrombosis [11], stimulates vascular endothelial cell growth [12] and also stimulates smooth muscle proliferation of the affected blood vessels [13]. It was also observed to be a carrier of oxidised phospholipids in the blood [14] (Fig 3). Serum concentrations of Lp(a) are genetically determined to a large extent by the polymorphic apo(a) gene on chromosome 6q27. The apo(a) kringle IV (K-IV) repeat polymorphism with 30 alleles generates apo(a) molecules ranging from 300 to 800 kDa, with an inverse relationship between apo(a) size and Lp(a) concentration [21-23] .

The sinister role of lipoprotein (a) has been well established in the development of Coronary and cerebro vascular disease by numerous studies across the globe [15-18]. The same can be true for Peripheral arterial disease, but it remains to be ascertained by more number of studies in native Indian population. Still, Lp (a) has not been approved widely as a conventional parameter in the lipid profile analysis, and it is not amenable to routine treatment with lipid lowering drugs. The normal reference value for Lp (a) is accepted as upto 30 mg/dL [19], but studies have shown varying cut-off values based on ethnicity and inter- individual characteristics [20]. Several population-based studies based on predominantly white European populations have found the prevalence of PAD to be between 6% and 18% over the age of 55 years. There has, however, been very little research into the prevalence of PAD in non-Caucasian populations [4]. An insight into these intriguing factors has led to the performance of this study, whose aims are: (i) To study the levels of lipoprotein (a) in atherosclerotic peripheral arterial disease patients from the south Indian population, and to assess the predictive power of Lp(a) as a marker of peripheral arterial disease, (ii)To determine the relation of other conventional risk factors of atherosclerosis with Lp(a) in peripheral arterial disease.

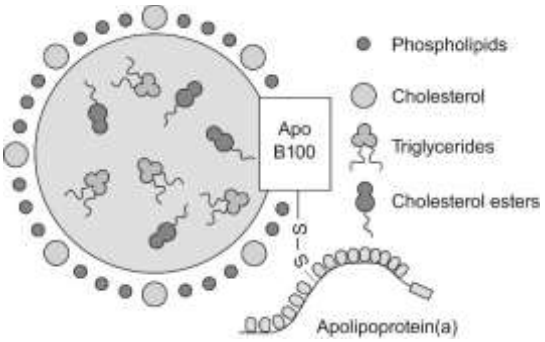

Fig 1: Lipoprotein (a) made up of LDL particle with apo B100 linked to apo (a) through disulphide bridges

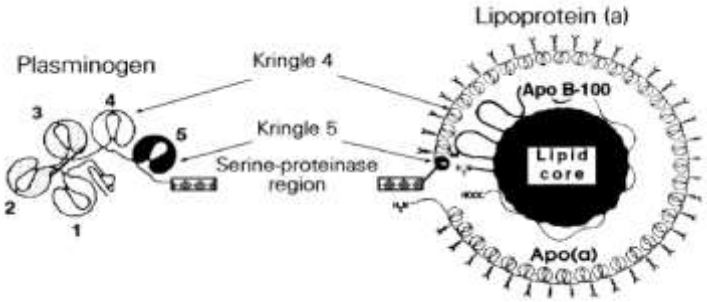

Fig 2: Structural homology of lipoprotein (a) with plasminogen

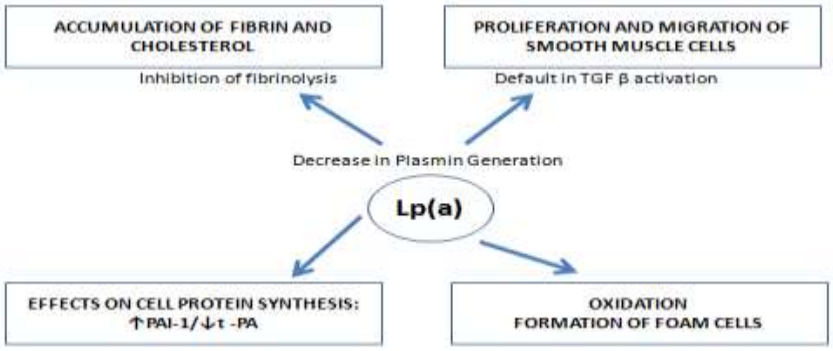

Fig 3: Pathogenic mechanisms of lipoprotein (a)

MATERIALS AND METHODS

After obtaining approval from the Institutional Ethical Committee, the study was conducted in the Department of Vascular surgery.

STUDY POPULATION

CASES

The study sample comprised 50 unrelated south Indian patients with symptomatic Peripheral Arterial Disease (47 males, 3 females) of Mean age 51.96 +/- 10.1 years. Inclusion criterion was ABPI (Ankle Brachial Pressure Index) less than 0.9 in the affected limb. Peripheral arterial disease other than that due to Atherosclerosis was excluded (eg. Thrombo angitis obliterans, autoimmune diseases). Patients diagnosed with malignancy, chronic liver disease, thrombotic tendencies and renal failure were also excluded from the study.

CONTROL SUBJECTS

Controls were recruited from Master health Check-up. Apparently healthy individuals matched for age and sex were included. All of them were free from symptoms and signs of peripheral arterial disease.

Sample collection and Processing

3 mL of blood was collected into a plain tube by Venipuncture after overnight fasting. The blood was allowed to clot and serum was separated by centrifugation. 0.5 mL of serum was stored in eppendorf tubes at -20^{o} C for analysis of Lipoprotein (a). The levels of total cholesterol, triglycerides and high density lipoprotein were measured in EM 360 fully automated analyzer by colorimetric methods using commercially available kits within 6 hrs of blood collection. Estimation of lipoprotein (a) was done with Lp (a) Turbilatex kit from Spin react using Immuno turbidimetry. LDL was calculated using Friedwald's formula (24):

LDL Cholesterol = Total Cholesterol -HDL Cholesterol – Triglycerides/5

STATISTICAL ANALYSIS:

The statistical software SPSS pc+ (Statistical Package for Social Science) was used for statistical analysis. Mean and Standard deviation were estimated from the sample each study group. The mean values were compared by students' t-test to calculate the p value. P value < 0.05 was considered significant. Chi square test was used for comparison of the variables between controls and cases. Pearson's Correlation analysis was used to correlate the levels of Lp (a) and other lipid parameters.

RESULTS

Table 1 shows the comparison of measured parameters between cases and controls. Mean BMI for cases with PAD (25.6 +/- 3.9) is significantly higher than the mean BMI for controls (23.3 +/- 1.9). ($p < 0.01$). Mean serum total cholesterol level in cases (157.5 +/- 17.7) is significantly higher than that of the controls (195.1 +/- 47.2) ($p < 0.01$). Mean serum Triglycerides in cases (190.1 +/- 47.7) is significantly higher than that of controls (113.2 +/- 17.9) ($p < 0.01$). Mean serum LDL in cases (119.7 +/- 44.2) is significantly higher than that of controls (90.1 +/- 18.3) ($p < 0.01$). Mean serum HDL in cases (37.4 +/- 4.7) is significantly lower than that of controls (44.5 +/- 3.4) ($p < 0.01$). Mean serum Lp(a) in cases (63.2 +/- 20.5) is significantly higher than that of controls (12.1 +/- 3.3) ($p < 0.01$). The means of the level of Lp(a) in patients with and without DM were compared in Table 2. There was no significant difference in the level of elevation of Lp(a) in these two groups so it can be inferred that Lp(a) contributes independently to the occurrence of PAD. Table 3 shows the correlation between the levels of the lipid parameters Total Cholesterol, Triglyceride, HDL and LDL with Lp(a) among cases and controls, respectively. There is a statistically significant positive correlation with TGL levels and Lp(a), negative correlation with HDL and Lp(a) among cases. The only significant correlation found among controls is between HDL and Lp (a), a negative one.

Table 1: characteristics of patients with pad and of controls

S.No	Particulars	Controls (n=50)	Cases (n=50)
1	Age in years	51.96	51.98#
2	Body Mass Index	23.37	25.69**
3	Total Cholesterol (mg/dL)	157.52	195.14**
4	Triglyceride (mg/dL)	113.20	190.06**
5	LDL (mg/dL)	90.10	119.68**
6	HDL (mg/dL)	44.59	37.47**
7	Lipoprotein(a) (mg/dL)	12.13	63.27**

Table 2: Comparison Of Levels Of Lp (A) Among Diabetics & Non Diabetics

	DM	**Mean**	**Std. Deviation**	**P value**
Lp(a)	Yes	68.33	19.32	0.157 #
	No	59.89	20.92	

Table 3: Correlation between other lipid parameters with Lp (a) in controls and cases (Pearson Correlation)

S.No	Parameter	Controls	Cases
1	Total Cholesterol	0.116	0.131
2	Triglyceride	0.213	0.552**
3	LDL	0.138	0.071
4	HDL	-0.433**	-0.468**

not significant; * significant ($p<0.05$); ** highly significant ($p<0.01$)

DISCUSSION

Peripheral arterial disease has been increasingly gaining importance as a major cause of debilitation and death. While there are voluminous records of studies done on coronary artery and cerebrovascular diseases, Indian studies on peripheral arterial disease appear to be relatively scarce, the dearth more apparent among the south Indian population. This study has attempted to determine the characteristics of atherosclerotic PAD and the risk factors in a south Indian population, the subjects mainly belonging to Tamilnadu, some of them having origin in Andhra Pradesh. But there did not seem to be any gross difference in the measured parameters between them, so they are evaluated as a single group of cases.

The gender distribution of cases in this present study is 47 males and 3 females, 2 of the females in the post menopausal age group. This clearly shows that male sex is in itself a risk factor for atherosclerotic PAD, estrogen being protective in women till menopause. The conventional risk factors like increased BMI, total cholesterol, triglycerides and LDL and decreased HDL are significantly higher in the cases, emphasizing their causative role. Hypertriglyceridemia seems to be more significant than hypercholesterolemia. This study has given importance to evaluate the association of lipoprotein (a) in atherosclerotic PAD. The levels of Lp (a) are increased to enormous proportions in cases than controls (63.3 vs 12.1 respectively). Lp (a) shows positive correlation with TGL and not total cholesterol or LDL in cases, and there is no significant correlation in controls, possibly due to the genetic makeup of the cases. Negative correlation is seen with HDL both in cases and controls, proving that HDL is protective. The possibility of a common genetic trait that influences the production of HDL and Lp (a) in a reciprocal manner cannot be omitted and needs to be evaluated in further studies, along with the pathogenic relationship between these two molecules. Similarly, the pathogenic relationship of Lp (a) with other markers of inflammation needs to be studied in detail to develop potential treatment strategies and drugs.

CONCLUSION

From the results of the study it can be concluded that, increased level of Lipoprotein (a) is an independent risk factor for atherosclerotic peripheral arterial disease and has a strong negative correlation with HDL cholesterol levels. Estimation of Lipoprotein (a) can be included in the routine analysis of lipid profile in patients with peripheral arterial disease. Studies on a larger scale can be undertaken in different parts of India and Asia to establish definite cut off values for Lp(a) according to the ethnicity and lifestyle. Genetic studies focusing on the gene pattern of lipoprotein (a), the apo A gene, and its influence over the levels of serum Lp(a) and other parameters like HDL and LDL, can be encouraged since it will give a clearer insight into the pathogenic relationship among them. Research aimed at developing drugs against the pathologic consequences of Lipoprotein (a) can be undertaken.

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