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ORIGINAL ARTICLE

# Lipoprotein (a) and its correlation to free radical nitric oxide in coronary heart disease subjects

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## Abstract

Lipoprotein (a) is comprised of low-density lipoprotein cholesterol which promote endothelial dysfunction and impair the effect of vasodilation by reducing the availability of nitric oxide by inhibiting the eNOS expression. Higher Lipoprotein (a) levels are linked with a higher risk of coronary heart disease. The objective of the study is to find the association between Lipoprotein (a) and nitric oxide levels in coronary heart disease. This cross-sectional study was conducted on subjects attending the Cardiology and medicine outpatient. Nitric Oxide and Lipoprotein (a) measured by ELISA method and Lipid Profile is measured using Auto Analyser AU480. Statistical analysis was done by using Student 't' test and Pearson correlation analysis for the comparison between two groups. The mean level of Low Density Lipoprotein level and Lipoprotein (a) in CHD was elevated and show a statistically significant difference (<0.05) compared to the normal healthy controls. And the mean Nitric Oxide levels were decreased significantly in CHD group compared to controls. Elevated level of Lipoprotein (a) an diminished production of Nitric oxide plays a major role in the progression of Coronary heart disease cascade.

**Keywords:** Coronary Heart Disease, High Density Lipoprotein, Low Density Lipoprotein, Lipoprotein, Lipoprotein (a) and Nitric Oxide.

## Introduction

Coronary heart disease is one of the most common form of heart disease caused by coronary atherosclerosis.[1] The most essential signs of Coronary heart disease are stable angina and acute coronary syndrome which comprise of unstable angina, myocardial infarction, heart failure, arrhythmia and unexpected cardiac death.[2]

Lp (a) is considered as a variant of Low Density Lipoproteins (LDL-C) act as an independent genetic risk indicator for Coronary heart disease.[3] It was discovered by Kare Berg in 1963. Lipoprotein (a) is an atherogenic lipoprotein synthesized and secreted by liver which comprises of cholesterol rich LDL-like particle and the specific protein apo (a), which is linked to apo B-lOO by Disulphide Bridge which differentiates it from LDL.[4] Half-life of Lipoprotein (a) in circulation is 3-4 days. Lp (a) in the pathogenesis of atherosclerosis was initially suggested by the presence of apo(a) in atherosclerotic lesions with apo(a)/apoB ratio. Lipoprotein (a) Inhibits fibrinolysis. Lp (a) level > 30mg/dL is associated with high risk (3 times more) for heart attacks. High Lp (a) level along with high LDL increases the risk of heart attacks by 6 times.[5] Lp (a) promote endothelial dysfunction over various mechanisms. Normal endothelium shows increased permeability as well as pro-coagulative and pro-inflammatory properties cause endothelial dysfunction implicated in the initial phases of atherosclerosis.[6] Lp (a) impair vasodilation through reducing nitric oxide (NO)

availability by inhibiting expression of eNOS.[7] Lp(a) stimulate the pro-atherogenic changes due to its relation to LDL particles, and also the capability for interaction with fibrin and tissue matrix constituents in arterial walls.[8] The accumulate on of Lp (a) in atherosclerotic plaques contribute to the growth of atheroma.[9] Lp (a) stimulate inflammatory progressions by prompting monocyte chemotactic action of vascular endothelial cells and stimulate pro-thrombotic developments by interfering with the action of plasminogen.[10] Lp (a) is interrelated to coronary heart disease due to foam cell formation by promoting oxidative and inflammatory processes in arterial wall.[11] In physiological conditions, NO is an essential molecule for endothelial function.[12] This endothelial-derived vasodilator is formed by the alteration of L-arginine into L-citrulline by endothelial nitric oxide synthase (eNOS) enzyme.[13] Nitric oxide acts as a significant factor of cardiovascular health.[14] Impaired NO production is cause for cardiovascular diseases.[15] Endothelial cells regulate basal vascular tone and reactivity by liberating a variation of vasoactive factors. The inability of endothelial cells to perform physiological functions leads to impaired function of the endothelium, known as endothelial dysfunction.[16] Dysregulation of several molecules contribute to endothelial dysfunction. Defect in nitric oxide (NO) synthesis or activity have major mechanism for endothelial dysfunction.[17] Endothelial dysfunction is an early occurrence in cardiovascular disease lead to decreased production of NO bioavailability and prostacyclin. This study was performed to elucidate the association between Plasma Nitric oxide and Lp (a) in progression of coronary heart disease. So our study hypothesized that rather than a mediator of vascular disease, Lp (a) is appears to be a marker and redirected as one of the promising risk aspects of atherosclerosis.

## Materials and Methods

This cross-sectional study was conducted from Jun 2019 to Dec 2019 at SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu, India on subjects attending the Cardiology and medicine outpatient. Totally 182 subjects were included who were age and sex match in the age group 30-55 years. 91 CHD subjects and 91 normal healthy subjects were selected as control. The control subjects were also taken from Master health check-up Programme and medicine OP. The study protocol was approved by the institutional ethical committee (ECN: 1513/ICE/2018).

### Inclusion Criteria

The CHD patients were recruited on the basis of proven coronary angiography.

### Exclusion Criteria

The subjects who were on treatment for diabetes, hypertension, renal failure, thyroid, arthritis, rheumatoid arthritis and acute / chronic infection patients.

All the patients registered in the study were clarified about the study and a written informed consent was taken. The demographic details, relevant history and anthropometric measurement were recorded. After overnight fasting Blood sample (5ml) was collected in sodium citrate and plain vacationer under aseptic precaution. 2ml of blood was taken for the measurement of Lipid profile (Total cholesterol by **Cholesterol Oxidase** method, Triglycerides by **Glycerol peroxidase** method, HDL-C and LDL-C by **Direct method** using Beckman Coulter Auto analyser (AU480) and the remaining 3ml of blood was allowed to clot for 30 minutes and then centrifuged at 2500 RPM for 10 minutes for the quantification of Nitric Oxide and Lp(a) was done by (Cayman) ELISA method.

### Measurement of Nitric Oxide

Serum NO was measured by using Griess reagent as nitrite/nitrate in CHD subjects and control. It is a two-step processing. Conversion of nitrate to nitrite is the first step which helps in utilizing nitrate reductase. Due to the addition of Griess Reagents in second step the nitrite is converted into deep purple azo compound. This azo chromophore perfectly determines $NO_2$-concentration. Absorbance are measured at 540 nm

## Statistical Analysis

All the data was analysed using Statistical Package for Scientific Studies (SPSS) version 16. The results

were represented as mean ± standard deviation (SD). Student's t-test was used to analyse the difference between the mean levels of various parameters. Correlation between various variables was assessed using Pearson's correlation equation. The p-value <0.05 was considered statistically significant.

## Results

Among the 182 Participant 91 CHD subject (58 males and 43 females) with average age 45.3 ± 9.4 years and 91 Healthy Control (40 males and 51 females) with average age of 39.8 ± 7.2. In CHD patients the mean level of BMI, Waist Circumference, Waist Hip Ratio, systolic blood pressure were significantly ($p < 0.05$) elevated when compared to controls are given in (Table 1).

The Mean levels of FBG, Total Cholesterol, Triglyceride, LDL-C and Lp (a) are significantly increased in subject with CHD compare to controls, were as the mean levels of HDL-C were decreased when compared to control. The Mean levels of NO are significantly decreased in patients with CHD compare with the controls.

Nitric oxide were positively correlated with FBG, HDL-C. The negative correlation was found in BMI, WC, WHR, Total Cholesterol, Triglyceride, LDL-C, ox-LDL were observed. The values were summarized in (Table 3).

In our study Lp(a) is positively associated with BMI, WC, WHR, Total Cholesterol, Triglyceride,

**Table 1** Demographic Profile of Coronary Heart Disease Subject and Healthy Controls

| Parameters | Controls (n = 91) | CHD patient (n = 91) | P-Value |
|---|---|---|---|
| **Mean age (years, mean ± S.E.M.)** | 39.8 ± 7.2 | 45.3 ± 9.4 | <0.0001*** |
| **Male Sex (%)** | 40 (37%) | 58(69%) | — |
| **Female Sex (%)** | 51(63%) | 43(41%) | — |
| **Body mass index (kg/m$^2$)** | 21.91 ± 0.37 | 23.47 ± 0.35 | <0.0001*** |
| **Waist circumference (cm)** | 90.9 ± 10.1 | 93.8 ± 9.6 | <0.04* |
| **Waist to hip ratio** | 0.94 ± 0.02 | 1.01 ± 0.01 | <0.0001*** |
| **Waist to height ratio** | 0.56 ± 0.01 | 0.62 ± 0.02 | <0.0001*** |
| **Systolic blood pressure, mm Hg** | 112.73 ±18.32 | 126.46 ±19.84 | <0.0001*** |
| **Diastolic blood pressure, mm Hg** | 77.69 ± 7.95 | 74.58 ±13.26 | <0.0001*** |

Values are expressed in Mean ± Standard Deviation. NS-Not significant,
*P value < 0.05 is considered significant, ***Very Highly significant, **Highly Significant.

**Table 2** Biochemical parameters of Coronary Heart Disease Subject and Healthy Controls

| Parameters | Controls (n = 91) (Mean ± SD) | CHD Patient (n = 91) (Mean ± SD) | P- Value |
|---|---|---|---|
| **FBG(mg/dl)** | 90.24 ± 4.18 | 94.29 ± 6.98 | NS |
| **Total Cholesterol (mg/dl)** | 169.20 ± 16.13 | 215.6 ± 34.27 | <0.0001*** |
| **TG (mg/dl)** | 82.74 ± 28.41 | 140.19 ± 60.71 | <0.0001*** |
| **HDL (mg/dl)** | 46.96 ± 9.4 | 37.64 ± 4.12 | <0.0001*** |
| **LDL (mg/dl)** | 106.54 ± 12.45 | 189.4 ± 27.46 | <0.0001*** |
| **VLDL (mg/dl)** | 17.26 ± 8.77 | 28.06 ± 12.14 | <0.0001*** |
| **TC/HDL-C Ratio** | 3.71 ± 0.70 | 6.17 ± 1.14 | <0.0001*** |
| **LDL-C/HDL-C Ratio** | 2.35 ± 0.53 | 4.22 ± 0.75 | <0.0001*** |
| **Nitric Oxide (µmol/L)** | 19.08 ± 4.74 | 12.97 ± 1.20 | <0.0001*** |
| **Lp(a) (mg/dl)** | 17.77 ± 3.56 | 35.80 ± 4.36 | <0.0001*** |

Values are expressed in Mean ± Standard Deviation.
*P value < 0.05 is considered significant, ***Very Highly significant, **Highly Significant.
NS-Not significant, FBG- Fasting Blood Glucose; TC- Total Cholesterol; TG- Triglyceride; HDL- High Density Lipoprotein; LDL- Low Density Lipoprotein; NO- Nitric Oxide; and Lp(a)- Lipoprotein (a)

**Table 3** Pearson Correlation Analysis between Lp(a) and Nitric Oxide in Coronary Heart Disease Subjects

| | Lp(a) | P- Value | Nitric Oxide | P- Value |
|---|---|---|---|---|
| **BMI** | 0.113[a] | <0.0001*** | –0.127[b] | <0.0001*** |
| **Waist Circumference** | 0.028[a] | <0.0001*** | –0.031[b] | <0.0001*** |
| **Waist Hip Ratio** | 0.302[a] | <0.0001*** | –0.317[b] | <0.0001*** |
| **FBG** | –0.109[b] | <0.0001*** | 0.078[a] | <0.0001*** |
| **Total Cholesterol** | 0.920[a] | <0.0001*** | –0.915[b] | <0.0001*** |
| **Triglyceride** | 0.155[a] | <0.0001*** | –0.143[b] | <0.0001*** |
| **HDL-C** | –0.144[b] | <0.002** | 0.134[a] | <0.002** |
| **LDL-C** | 0.994[a] | <0.0001*** | –0.991[b] | <0.0001*** |
| **VLDL-C** | 0.153[a] | <0.0001*** | –0.104[b] | <0.0001*** |
| **Cardiac Risk Ratio- I** | 0.632[a] | <0.0001*** | –0.998[b] | <0.0001*** |
| **Cardiac Risk Ratio- II** | 0.740[a] | <0.0001*** | 0.766[a] | <0.0001*** |
| **Lp(A)** | — | — | –0.987[b] | <0.0001*** |
| **Nitric Oxide** | –0.987[b] | <0.0001*** | — | — |

[a]Positive Correlation
[b]Negative Correlation

LDL-C, TC/HDL-C Ratio and LDL/HDL-C Ratio. The negative correlation was found in FBG, HDL-C and NO were observed. The values were summarized in (Table 3).

## Discussion

In premature atherosclerosis, diminished function of endothelium is the first sign of vascular variations.[18] An elevated plasma concentration of Lp(a) is denoted as an self-regulating threat aspect for coronary heart disease and peripheral atherosclerosis.[19] In the present study we hypothesized that the elevated plasma Lp(a) levels may be intermediated through endothelial dysfunction. In present study serum level of Lp(a) and LDL cholesterol were significantly elevated in CHD subjects when compared to controls. Subjects with high LDL-C, showed a decreased NO levels compared to controls. The cholesterol contained within HDL is inversely linked with risk of coronary heart disease and is a strategic factor of predicting cardiovascular risk. In humans and other primates Lp(a) accumulates in the atherosclerotic lesions in vessel wall. Lipoprotein (a) binds to various proteins in the extracellular matrix through its apo (a) component or LDL moiety bind the proteins with a higher affinity than LDL.[20] The levels of lipoprotein (a) is found to correlate with the severity score in coronary Angiogram. Lp (a) remains to be one of the possible culprits for the development of premature CHD in Indians. A strong genetic predisposition of Lp (a) holds the upcoming generation of the affected population at risk for developing CHD in future.[21] Vinod Chacko et al., stated that higher lipoprotein (a) levels were found to be an inherited risk aspect for premature CHD in asian countries.[22] Malaguarnera et al., found increased levels of lipoprotein (a) levels in coronary heart disease subjects when compared to control. Lp(a) reduce the availability of NO due to excess production of free radical which contribute to CHD.[23]

In our study Lipoprotein (a) is found to be the independent predictor for coronary artery disease along with high LDL. Occurrence of apo (a) rises the concentration of Lp (a) linked with LDL-C and decreases its affinity for the LDL receptor. Many studies strongly revealed that apo B could be superior indicator of threat for vascular disease. NO released by endothelial cells maintain a vasodilator role in the cardiovascular system.[24] Endothelial dysfunction occurs with altered prostaglandin-$i_2$ and nitric oxide biosynthesis. Su et al., stated that hypercholesterolemia is a crucial pathogenic aspect of endothelial dysfunction caused by production of impaired endothelial nitric oxide.[25] Endothelial cells bind with Low density lipoprotein cholesterol in blood conquer endothelium and gets oxidized by free radicals and initiates inflammatory response resulting in lipid peroxidation.[26] Loss of endothelial integrity, characterized

by diminished production of NO, is accepted as an essential feature in the development of atherosclerosis and thrombosis. Exogenous and endogenous NO prevents vascular smooth muscle cell (VSMC) proliferation and migration. Reduced bioavailability of NO and reduced nitric oxide bioactivity may assist vascular inflammation that lead to lipoproteins oxidation and formation of foam cell implicated in the development of coronary heart disease.[27] Albers et al., found that Lp(a) levels are significantly elevated in younger age groups of MI subjects.[28] Vasisht et al., found a strong relation between the elevated Lp(a) and CHD.[29] The early prediction of Lp(a) is useful in young subjects with premature coronary heart disease without established risk factors like diabetics, hypertension and subjects with a family history of coronary heart disease.

## Conclusion

The study concludes that there is a strong association between Lp(a) and NO in the development of CHD. Lipoprotein (a) is an independent genetic risk factor for the development of coronary heart disease. Lipoprotein (a) rises the threat of atherogenicity 10 times equated to LDL. Old-style risk features are inadequate for risk prediction in subjects with CHD. Because Lp(a) and Nitric oxide levels are not currently measured with a routine lipid profile because methods of measurement are technically challenging. No organization to date has undertaken the challenge to standardize NO and Lp(a).

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## Conflict of Interest

No conflict of Interest

## Financial support

Nil

## Author's Contributions

All authors have made important contribution to the research work reported in this manuscript.

Meera Shivasekar – Designing and drafting of the study and critical revision of the article

Thirunavukkarasu Jaishankar – data collection, data analysis and interpretation

Vinodhini V.M – Final inputs and approval of the article to publish.

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