



## Emerging Risk Markers; Nitric Oxide and Oxidized Low-Density Lipoprotein in Predicting Coronary Heart Disease in Young subjects

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### ABSTRACT

NO is a free radical gas, identified as Endothelial Derived Releasing Factor (EDRF) and synthesized from L-arginine which plays a dynamic role in defence against onset and development of coronary heart disease. Decreased availability of nitric oxide is one of the important cause for the pathogenesis of atherosclerosis. An accumulation of Low Density Lipoprotein cholesterol (LDL) in the intimal wall is the early step in atherosclerosis. Reduced NO level in the endothelium makes it vulnerable and increase the passage of leukocyte and undergo LDL oxidation in the sub endothelial space which ultimately lead to coronary heart disease. The aim of the study is to assess role of Nitric Oxide and oxidized LDL in CHD subjects. This cross-sectional study was conducted in SRM Medical college Hospital and Research centre on subjects attending the Department of Cardiology and Medicine OP. The study was conducted on 194 subjects in age group <40 years and were age and sex matched. After overnight fasting blood samples were collected for analysis for Lipid Profile and Nitric oxide and ox-LDL. Nitric Oxide and ox-LDL measured by ELISA method. Statistical analysis was done using Student 't' test and Pearson correlation analysis for the comparison between two groups. The mean level of Nitric Oxide were decreased ( $12.97 \pm 1.20$ ) significantly in CHD group compared to controls ( $19.08 \pm 4.74$ ) ( $p < 0.001$ ). And Oxidized LDL showed significant increase ( $41.53 \pm 8.72$ ) in CHD group when compared to controls ( $16.73 \pm 3.55$ ) ( $p < 0.001$ ). Reduction in the levels of NO in the arterial wall impairs endothelial function. The study concludes that there is a strong association between NO and ox-LDL in progression of CHD. Interestingly, these analyses can help as diagnostic and monitoring markers in young CHD patients.



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### INTRODUCTION

Coronary Heart Disease is one of the major cause of death in India. CHD is generally stated as a multifactorial disorder related to the genetic and environmental aspect ([Rajeev-Gupta et al., 2016](#)). Nitric oxide and ox-LDL are the antagonist and they are the key mediators for initiation, progression and complications in all stages of atherosclerosis: oxLDL have pro-atherogenic and NO have antiatherogenic functions on vascular endothelium ([Marchio et al., 2019](#)). Nitric oxide is a free radical formed from L-arginine by altering into L-citrulline by nitric oxide synthase enzymes ([Forstermann et al., 2012](#)) which

act as a crucial modulator of vascular disease. Damage to the endothelium caused by the excess of lipoproteins which are promoted by atherosclerosis cause reduction in the bioactivity of endothelial NO synthase (eNOS) (Seinosuke-Kawashima *et al.*, 2004). When endothelium turns more permeable to lipoproteins, a variation of factors which decrease the availability of NO includes the availability of substrate L-arginine, increased concentration of circulating inhibitor ADMA, changed levels of expression in eNOS, signal transduction reducing eNOS activation, decreased tetrahydrobiopterin (BH4) availability (Bendall *et al.*, 2014). Lipoproteins loses its cell-repellent quality when it moves below the endothelial layer. Retention of LDL in the intima, which undergoes oxidative modification by the action of oxygen free radical caused due to inflammatory cells moved into the vascular wall (Alique *et al.*, 2015). ox-LDL up was taken by macrophages via phagocytosis resulting in the formation of foam cell (Mundi *et al.*, 2018). Decreased production of NO suggested as major mechanisms of endothelial dysfunction, which contribute to the progression 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 194 subjects were included who were age and sex match in the age group <40 years. 97 CHD subjects and 97 normal healthy subjects were selected as control. The control subjects were also taken from Master health check-up Programme and medicine OP in SRM Medical College Hospital and Research Centre, Chennai, Tamil Nadu, India. This study was approved by the institutional ethical committee at SRM Medical College Hospital and Research Centre (ECN: 1513/ICE/2018). From all participants written, informed consent was collected at the time of enrolment.

### Inclusion Criteria

The CHD patients were selected on the basis of coronary angiography and with chest pain. The control group consists of persons with no clinical and ECG evidence of CHD and negative history of the past event of CHD or stroke, DM, hypertension, smoking, Dyslipidemia, and family history of CHD.

### Exclusion Criteria

Malignant tumors, liver and kidney damage, thyroid disease, Nephrotic syndrome, Patients who have taken lipid-lowering drugs.

## Anthropometric Measurement

The baseline examination included medical history, health habits and family history of CHD and diabetes. The physical examination consisted of 12-lead resting electrocardiogram. The anthropometric measurement, including Weight (Kg), height (meters), waist circumference (cm) and hip circumference (cm), BMI and waist-hip circumference ratio, were calculated. Systolic and diastolic arterial blood pressures were measured.

After overnight fasting Blood sample (5ml) was collected in sodium citrate and plain vacutainer under aseptic precaution. 2ml of blood was taken for the measurement of Glucose and Lipid profile include (Total cholesterol by Cholesterol Oxidase method, Triglycerides by Glycerol peroxidase method, HDL-C and LDL-C by Direct method high-density lipoprotein (HDL) cholesterol (HDL-C) were measured 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 (Griess Method) and oxidized LDL was done by ELISA method.

## Statistical Analysis

Using Statistical Package for Scientific Studies (SPSS) version 16 all the data was analysed. The results were denoted as Mean  $\pm$  Standard Deviation. The difference between the mean levels of various parameters were analysed by Student's t-test. Correlation between various variables was assessed using Pearson's correlation equation. The p-value <0.05 was considered statistically significant.

## RESULTS

Among 194 subjects, 97 are control (40 males and 57 females) with average age of  $32.8 \pm 4.7$  and 97 CHD patient (58 males and 39 females) with average age  $35.16 \pm 4.28$  years. In the present study the level of BMI, Waist Circumference, Waist Hip Ratio, systolic blood pressure were significantly ( $p < 0.05$ ) higher in CHD patients as compared to controls as Shown in [Table 1].

The study shows FBG, Total cholesterol, Triglyceride, LDL-C, VLDL-C, LDL/HDL ratio, Total Cholesterol/HDL ratio. The mean levels of HDL-C levels not significantly associated among the two groups in subject with CHD [Table 2].

Plasma Nitric Oxide values were significantly decreased ( $12.77 \pm 1.37$ )  $\mu\text{mol/L}$  in CHD subject

**Table 1: Demographic characteristics of the study subjects**

Parameters	Controls (n=97)	CHD patient (n=97)
Mean age (years, mean $\pm$ S.E.M.)	32.8 $\pm$ 4.7	35.16 $\pm$ 4.28
Male Sex (%)	40 (41.23%)	58 (59.79%)
Female Sex (%)	57 (58.78%)	39 (40.2%)
Body Mass Index (BMI) (kg/m <sup>2</sup> )	21.91 $\pm$ 0.37	24.03 $\pm$ 0.19
Waist Circumference (WC) (Cm)	90.9 $\pm$ 10.1	98.8 $\pm$ 4.3
Waist Hip Ratio (WHR)	0.94 $\pm$ 0.02	1.05 $\pm$ 0.03
Waist to height ratio	0.56 $\pm$ 0.01	0.65 $\pm$ 0.01
Systolic Blood Pressure (SPB)	109.73 $\pm$ 18.32	122.26 $\pm$ 13.95
Diastolic Blood Pressure (DBP)	77.69 $\pm$ 6.95	76.16 $\pm$ 3.47
Family history of CHD		
Yes	0	46 (47.42%)
No	97(100%)	51 (52.57%)

BMI-Body Mass Index, WC- Waist Circumference, WHR- Waist Hip Ratio, SBP- Systolic Blood Pressure DBP- Diastolic Blood Pressure

**Table 2: Comparison of Lipid profile in Coronary Heart Disease Subject and Healthy Controls**

Parameters	Controls (n=97)	CHD patient (n=97)	P-Value
Fasting Blood Glucose (FBG)	90.24 $\pm$ 4.18	94.29 $\pm$ 6.98	NS
Total cholesterol (mg/dl)	168.8 $\pm$ 16.3	239 $\pm$ 41.42	<0.0001***
Triglyceride (mg/dl)	84.6 $\pm$ 30.5	159.7 $\pm$ 69	<0.0001***
HDL	46 $\pm$ 9	37.83 $\pm$ 4.25	NS
TC/HDL Ratio	3.71 $\pm$ 0.70	6.50 $\pm$ 1.36	<0.0001***
LDL/HDL Ratio	2.35 $\pm$ 0.53	4.41 $\pm$ 0.90	<0.0001***
LDL (mg/dl)	106.4 $\pm$ 12.59	164.64 $\pm$ 27.32	<0.0001***
VLDL (mg/dl)	17.26 $\pm$ 8.77	34.08 $\pm$ 14.29	<0.0001***
Nitric Oxide ( $\mu$ mol/L)	19.08 $\pm$ 4.74	12.77 $\pm$ 1.37	<0.0001***
ox-LDL (U/L)	16.73 $\pm$ 3.55	42.82 $\pm$ 10.03	<0.0001***

FBG-Fasting Blood Glucose; TC- Total Cholesterol; TG- Triglyceride; HDL- High Density Lipoprotein; LDL- Low Density Lipoprotein; NO- Nitric Oxide; ox-LDL-Oxidized Low Density Lipoprotein

Values expressed as Mean $\pm$ SD. Students t-test. \*P value < 0.05 is considered significant, NS-Not significant, \*\*\*Very Highly significant, \*\*Highly Significant

when compared (19.08 $\pm$ 4.74)  $\mu$ mol/L with controls (P < 0.001). Serum ox-LDL show a statistically significant increase (42.82 $\pm$ 10.03) U/L in CHD subject when compared (16.73 $\pm$  3.55) U/L with controls (P < 0.001). A significant positive correlation was also observed between ox-LDL and total cholesterol. Plasma ox-LDL level shows a positive correlation with LDL-C and Triglyceride clearly confirms that LDL in CHD patients is more vulnerable to oxidative modification than LDL from a healthy control. Nitric Oxide positively correlated with BMI (r = 0.160), Triglyceride (r = 0.036), HDL-C (r = 0.046). And Nitric Oxide negatively correlated with Waist Circumference (r = -0.035), Waist Hip Ratio (r = -0.027), FBG (r = -0.150), Total Cholesterol (r = -0.812), LDL-C (r = -0.999), VLDL-C (r = -0.113), Cardiac Risk Ratio- I (r = -0.059), Cardiac Risk

Ratio- II (r = -0.066) and ox-LDL-C (r = -0.995) [Table 3]. Oxidized LDL positively correlated with BMI (r = 0.111), Waist Circumference (r = 0.035), Waist Hip Ratio (r = 0.296). Triglyceride (r = 0.145), Total Cholesterol (r = 0.920), LDL-C (r = 0.996), VLDL-C (r = 0.144), Cardiac Risk Ratio- I (r = 0.634), Cardiac Risk Ratio- II (r = 0.743). And Oxidized LDL negatively correlated with HDL-C (r = -0.148), Nitric Oxide (r = -0.995) [Table 3].

## DISCUSSION

Elevated level of LDL-C associated with increased inflammatory events in patients who are positively correlated with the severity of Disease (Paoletti *et al.*, 2004). Impairment in production of NO and elevated levels of oxidized LDL can be helpful to

**Table 3: The Pearson correlations analysis between Nitric Oxide and oxidized LDL with other biochemical parameters in subjects with CHD**

	NO	Ox-LDL	P- Value
BMI	0.160 <sup>a</sup>	0.111 <sup>a</sup>	<0.0001***
Waist Circumference	-0.035 <sup>b</sup>	0.035 <sup>a</sup>	<0.0001***
Waist Hip Ratio	-0.027 <sup>b</sup>	0.296 <sup>a</sup>	<0.0001***
FBG	-0.150 <sup>b</sup>	-0.106 <sup>b</sup>	<0.0001***
Total Cholesterol	-0.812 <sup>b</sup>	0.920 <sup>a</sup>	<0.0001***
Triglyceride	0.036 <sup>a</sup>	0.145 <sup>a</sup>	<0.0001***
HDL-C	0.046 <sup>a</sup>	-0.148 <sup>b</sup>	<0.002**
LDL-C	-0.999 <sup>b</sup>	0.996 <sup>a</sup>	<0.0001***
VLDL-C	-0.113 <sup>b</sup>	0.144 <sup>a</sup>	<0.0001***
Cardiac Risk Ratio- I	-0.059 <sup>b</sup>	0.634 <sup>a</sup>	<0.0001***
Cardiac Risk Ratio- II	-0.066 <sup>b</sup>	0.743 <sup>a</sup>	<0.0001***
ox-LDL	-0.995 <sup>b</sup>	-	<0.0001***

a- Positive Correlation

b- Negative Correlation

predict the progression of coronary heart disease. Therefore, our study gives more information on the association between ox-LDL and Nitric oxide for predicting the progression of coronary heart disease.

HDL is inversely related to the risk of coronary heart disease and also act as a key component for predicting cardiovascular risk (Rader *et al.*, 2014).

NO is an important regulatory molecule for cardiovascular functioning in health (Strijdom *et al.*, 2009). For preventing atherogenesis, atheromatous plaques formation the release of NO plays a major role (Matthys *et al.*, 1997). Nitric Oxide deficiency contributes to the pathogenesis of CHD (Richard.O.Cannon *et al.*, 1998). Plasma NO levels were significantly lower in subject with coronary heart disease. The physiological levels of NO are essential to maintain the normal, vasodilatory and non-inflammatory phenotype in the intimal wall (Rajendran *et al.*, 2013). A main outcome of the study show that the level of NO is negatively correlated with circulating ox-LDL.

Elevated levels of oxidized LDL, formed within the arterial wall, are commonly related to the atherogenic profile (Witztum *et al.*, 1991).

Uptake and accumulation of oxidatively modified LDL by macrophages in the vessel wall, initiate a wide range of bioactivities followed by migration into the intima lead to foam cell formation (Gleissner *et al.*, 2007) due to overproduction of reactive oxygen species via endothelial cells under oxidative modifications would be crucial in the progression of coronary heart diseases such as endothelial dysfunction and plaque disruption (Singh *et al.*,

2002). Endothelial cells which bind to LDL when it is activated by injury; and these cells are attached to macrophages and generate free radicles which oxidize the LDL. By endothelial cells, oxidized LDL stimulates the release of growth factors which are cytotoxic to endothelial and smooth muscle cells cause endothelial cell dysfunction. An elevated level of ox-LDL resulting in reduced NO bioavailability by combined effects of direct elimination and decreased production of NO. This NO reduced bioavailability which compromises all anti-atherogenic functions of the endothelium. Studies stated that Hyperlipidaemia can directly damage the endothelial cell function by increasing free radical production, which accelerate nitric oxide decay and reducing its vasodilator activity (Phaniendra *et al.*, 2015). Ox-LDL which reduce the availability of NO due to excess production of free radical superoxide (O<sub>2</sub><sup>-</sup>). Superoxide inactivates NO producing peroxynitrite (OONO<sup>-</sup>), causing injury to the coronary endothelium. Free radicals and NO initiate the lipid peroxidation in LDL-C, which contribute to the progression of CHD (Sozmen *et al.*, 2016). More studies stated that Ox-LDL is the major cause for hyperlipidaemia which triggers CHD (Jing-yi *et al.*, 2018). Thus the assessment of plasma Nitric Oxide is the essential strongest predictor of future risk of CHD. Reduction in NO is an early stage of the disease contributes to CHD.

## CONCLUSIONS

The study concludes that the level of Oxidized LDL cholesterol may alter the production of Nitric oxide. Therapeutic tactics in the preventing and treating

the subject with CHD based on enlightening NO production and lowering the level of circulating ox-LDL may become a challenge for future studies.

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The authors declare that they have no funding support for this study.

#### Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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