



## Correlation between HDL-C and Nitric Oxide in Normal Healthy Population

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### ABSTRACT

Prevention of coronary artery disease (CAD) and reduction of its mortality and morbidity remains a major public health challenge throughout the world. Nitric oxide (NO) plays a pivotal role in CAD (coronary artery diseases). This study was undertaken to show the correlation between HDL & NO in normal healthy population since decreased NO bioavailability is a key feature of all classic risk factors for Atherosclerosis. A cross sectional study was done with sample size 60. In this study it was found that lower Nitric oxide is significantly associated with higher level of HDL with  $P < 0.001$ . We conclude that constantly low HDL-C concentration is related with endothelial dysfunction and increased oxidative stress in healthy young men, consistent with the idea that HDL particles may protect endothelium and inhibit the oxidation of LDL. These findings may offer insight into increased atherosclerosis associated with low HDL-C levels.

**Key Words-** Nitric oxide, HDL-C, Atherosclerosis.

### INTRODUCTION

Prevention of coronary artery disease (CAD) and reduction of its mortality and morbidity remains a major public health challenge throughout the world. Over the last decade, a remarkable burst of evidence has accumulated, offering the new perspective that nitric oxide (NO) plays a pivotal role in CAD (coronary artery diseases). The risk of atherosclerosis is inversely related to circulating levels of high-density lipoprotein cholesterol (HDL-C) <sup>(1)</sup> and the Framingham

Heart Study demonstrated that the association is independent of low-density lipoprotein (LDL) cholesterol. In addition, clinical trials with agents that increase HDL show that elevations in the lipoprotein decrease the incidence of cardiovascular events <sup>(2)</sup>. Each 1mg/dl increase in HDL causes 2-4% reduction in the risk for CAD. HDL promotes NO production by stimulating eNOS (endothelial nitric oxide synthase) by various mechanisms <sup>(3)</sup>. The physiological actions of NO range from mediating vasodilatation,

neurotransmission, inhibition of platelet adherence/aggregation and the macrophage and neutrophil killing of pathogens<sup>(4)</sup>. The high rate of production and broad distribution of sites of production of .NO, combined with its facile direct and indirect reactions with metalloproteins, thiols and various oxygen radical species, assures that .NO will play a central role in regulating vascular physiologic and cellular homeostasis as well as critical intravascular free radical and oxidant reactions.

This study was undertaken to show the correlation between HDL& NO in normal healthy population since decreased NO bioavailability is a key feature of all classic risk factors for Atherosclerosis. Identification of alteration in NO bioavailability is beneficial in targeting asymptomatic individuals who are at risk for cardiovascular disorders. With further investigation and greater depth of understanding these mechanisms may be harnessed to provide new prophylactic and therapeutic strategies to combat atherosclerosis.

## MATERIALS AND METHODS

A cross sectional study was done with sample size 60. Fasting blood samples were collected from cases with inclusion criterion of healthy subjects with normal lipid profile, age between 25- 40years of both sex. The exclusion Criteria were subjects with abnormal lipid profile, diabetes mellitus,

liver diseases, renal and respiratory diseases, hypertension and coronary artery disease, Smoking and alcohol intake, and drugs altering the lipid profile like B-blockers, thiazide, prazosin and steroids. 5ml blood was collected in yellow vacutainer tube. Blood was allowed to clot, centrifuged and serum was transferred in eppendorff's tube. HDL-C, Total cholesterol and triglyceride was estimated immediately on fully automated analyzer<sup>(5,6)</sup>. Serum nitric oxide was estimated by Kinetic Cadmium-Reduction Method<sup>(7)</sup>.

## RESULT

The serum nitric oxide level has a mean±SD value of 26.43±13.99. The mean ±SD for HDL-C was 47.52±5.86, 39.24±4.76, 36.20±2.74 and 37.00±2.00, when nitric oxide levels were < 12.5, 12.5-25, 25- 50 and >50 with a significant p value of < 0.001. The Pearson correlation between NO and Total cholesterol, triglycerides and LDL do not give a significant value where as with HDL the Pearson correlation is -0.579 and is significant. We can infer that lower Nitric oxide is significantly associated with higher level of HDL with P<0.001.

**Table 1:** Mean values of total cholesterol, Triglycerides, LDL, HDL according to different levels of Nitric oxide

Lipid parameters	Nitric Oxide				P value
	<12.5	12.5-25.0	25.0-50.0	>50.0	
Total cholesterol	156.76±18.50	153.48±21.24	146.47±26.78	137.67±37.50	0.607
Triglycerides	113.57±28.92	115.00±37.02	115.67±25.99	110.67±15.31	0.976
LDL-C	82.24±23.48	88.61±24.58	86.80±26.34	81.33±35.52	0.868
HDL-C	47.52±5.86	39.24±4.76	36.20±2.74	37.00±2.00	<0.001**

Analysis of Variance with Welch robust analysis for equal variance

**Table 2:** Correlation of levels of HDL with levels of Nitric oxide

HDL	Nitric Oxide			
	<12.5	12.5-25.0	25.0-50.0	>50.0
31-40	3(5.0%)	13(21.7%)	14(23.3%)	3(5.0%)
41-50	13(21.7%)	8(13.3%)	1(1.7%)	0
51-60	5(8.3%)	0	0	0
Total	21(35.0%)	21(35.0%)	15(25.0%)	3(5.0%)
Inference	Lower Nitric oxide is significantly associated with higher level of HDL with P<0.001**			

Statistical software: The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1 and Systat 12.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc

## DISCUSSION

Nitric oxide produced by endothelial cells regulates vasomotor tone and inhibits smooth muscle cell proliferation and leukocyte adhesion. Under pathological conditions, however, reactive nitrogen species derived from NO may injure vascular tissue. The pathway may be the rapid reaction of NO with superoxide, which could generate the potent oxidizing intermediates ONOO.

NO oxidizes proteins to form 3-nitrotyrosine, also oxidizes lipid moieties of LDL, converting the lipoprotein to form that is recognized by the macrophages scavenger receptor<sup>(8)</sup>. Unregulated uptake of such modified lipoprotein may play a role in cholesterol accumulation by macrophages, a critical early step in atherogenesis.

It has been demonstrated that HDL-C is oxidized by RNS in vivo; Lipoproteins are nitrated in vivo. HDL is a specific target for nitration in the human artery wall. Thus, nitrated HDL may represent a previously unsuspected biochemical link between inflammation, nitrosative stress & atherogenesis. The elevation in cytokine levels in CAD would increase iNOS expression, and such markers of inflammation predict vascular damage<sup>(9)</sup> Whereas endothelial NOS is the major NO-generating isoform in normal coronary arteries, iNOS may play a more important role in arteries with existing atherosclerotic changes. In these vessels, endothelial NOS is more likely to be depressed due to endothelial cell dysfunction. Activation of

iNOS may then become the major source of vascular wall bioactive NO. Although iNOS is classically regarded as requiring induction before it appears, it is quite likely that it could be expressed in a continuous manner in these diseased tissues, since cytokine stimuli may be there all the time. Such 'constitutive' iNOS expression not only should help in vasodilatation of these diseased vessels, but it could also play a role in remodelling.

In this study it was found that HDL correlated negatively with NO level in serum. Low HDL-C is an independent risk factor for endothelial dysfunction<sup>(10)</sup>. Quiescent endothelium produce NO which acts as vasodilator but active endothelium produce NO which act as ROS & promote atheroma<sup>(11)</sup>. NO deriving from endothelium is oxidized or participates in nitrosylation reaction. NO activity is the result of the balance between its production by NOS & its inactivation by oxyfree radicals.

The present study shows that in low normal (31-40) HDL population, No level was found to be high & in high normal (41-50) HDL population, NO level was found to be low. The reasons for that may be low normal HDL population the endothelium is dysfunctional & becomes active producing more NO which promote production of atheroma.

Another reasons may be due to presence of inducible form of NOS which is stimulated by

cytokines and produce much larger quantities of NO than other isoform because of active endothelium. Our observations indicate that reactive nitrogen species oxidize HDL in the human artery wall. Nitrated HDL also circulates in blood, and our preliminary studies suggest that humans suffering from clinically significant atherosclerosis contain elevated levels of the oxidized lipoprotein in their plasma. The detection of 3-nitrotyrosine in HDL isolated from vascular lesions raises the possibility that NO, by virtue of its ability to form reactive nitrogen intermediates, may promote atherogenesis.

Low level of nitric oxide concentration may be explained either due to decreased bioavailability of .NO due to either decreased expression of the eNOS, decreased substrate availability, presence of an endogenous eNOS inhibitor. We conclude that constantly low HDL-C concentration is related with endothelial dysfunction and increased oxidative stress in healthy young men, consistent with the idea that HDL particles may protect endothelium and inhibit the oxidation of LDL. These findings may offer insight into increased atherosclerosis associated with low HDL-C level.<sup>(12)</sup>

Further study is needed to understand the gene expression and enzyme activity of ecNOS and their association with genotypes.

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