Study on Role of Nitric Oxide and High Sensitive C-reactive protein in subjects with Coronary Heart Disease

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ABSTRACT
Nitric Oxide (NO) where produced by endothelial nitric oxide synthase (eNOS) enzyme which are inhibited by C-reactive protein (CRP) which causes endothelial dysfunction and cardiovascular events. In the current study, we evaluated the association of NO with hs-CRP in subjects with coronary heart disease. This Case-Control study was conducted 60 CHD patients and 60 healthy controls in age group of 30 to 55 years at SRM Medical College Hospital and Research Centre on subjects attending the Cardiology and medicine OP. Blood samples were collected after overnight fasting for analysis of Lipid Profile, High sensitive C-reactive protein. Nitric Oxide and High sensitive C-reactive protein is measured by ELISA method and Lipid Profile is measured using Auto Analyzer AU480. Statistical analysis was done using Student ‘t’ test and Pearson correlation analysis used to the variable between two groups. The mean level of LDL-C (161.9±27.46) and hs-CRP (6.80±1.35) were significantly elevated in CHD subjects when compared to the normal healthy controls. And the mean level of Nitric Oxide (12.97±1.20) were decreased significantly in CHD group when compared to controls. Increased oxidative stress associated with low grade inflammation lead to diminished bioavailability of nitric oxide.

INTRODUCTION
Nitric oxide is a free radical gas degraded in few seconds, involving one atom of nitrogen (N) and one atom of oxygen (O). NO is produced from amino acid L-arginine by enzymatic action of endothelial nitric oxide synthase (eNOS). Endothelial NOS (eNOS) enzyme produces NO in blood vessels and epithelial cells (Forstermann and Sessa, 2012). NO act as a biological messenger and termed as a potent relaxant of peripheral vascular smooth muscle (Thomas, 2015). Vascular endothelium produced nitric oxide is important in blood flow regulation. Impaired production of NO occurs in different altered disease states, adversely affect the vascular functions and blood flow (Loscalzo and Jin, 2010). Reduction in bioactivity of endothelial NO synthase (eNOS), damage the endothelium caused by excess of lipoproteins persuaded by atherosclero-
sis (Kawashima and Yokoyama, 2004). Decrease NO bioavailability is the essential factor common to coronary heart disease (Rajendran et al., 2013). Variation of factors which decrease availability of NO includes availability of substrate L-arginine, increased concentration of circulating inhibitor ADMA, changed levels of expression in eNOS, signal transduction reducing eNOS activation, decreased terahydrobipterin (BH₄) availability (Bendall et al., 2014). When endothelium turn more permeable to lipoproteins, it move underneath the layer of endothelium and loses its cell-repellent quality. Retention of LDL-C in intima which undergoes oxidative modification when inflammatory cells move into the endothelial wall (Alique et al., 2015). Nitric oxide is a significant biomarker of inflammation and oxidative stress (Ho et al., 2013).

The level of CRP rise drastically during inflammatory process (Singh et al., 2008). The elevated concentration of hs-CRP directly implies subclinical inflammation in an individual. The elevated concentration of CRP is released by liver by interleukin-6 stimulation and also produced in atheromatous lesions (Shrivastava et al., 2015). American Heart Association stated on inflammatory marker that identified hs-CRP as optimal inflammatory biomarker to estimate risk of CHD. CRP plays a major role in pathogenesis of atherosclerosis. Any inflammatory changes stimulate an acute phase response by macrophage, endothelial cells, adipocyte to secrete cytokines and chemokines. The cytokines regulate the production of CRP. The pathological process of atherosclerosis is responsible for most cause of cardiovascular disease (Paffen and Demaat, 2006).

Endothelial dysfunction well-defined as failure of vascular endothelium to function its normal role in vasodilatation. In functioning of endothelium, inflammatory response play an important role due to its endogenous and exogenous affectors, and the balance between endothelium derived contracting and relaxing factor get disturbed in endothelial dysfunction (Thompson et al., 2011).

**Exclusion Criteria**

The patients with the acute coronary syndrome, cardiomyopathy, and chronic disease like liver failure, cancer patient, heart failure, pregnancy, cardiovascular accidents, and serious systemic illness are excluded from the study.

All the patients registered were explained 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 analyzer (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 by UV Spectrophotometer (Cayman) and hs-CRP was measured by ImmunoTurbidometry in Marketable ELISA Kit.

**Statistical Analysis**

Data’s were evaluated using Statistical Package for Scientific Studies (SPSS) version 16. The results were denoted as mean ± standard deviation. For analyzing the difference between the mean levels of various parameters the Student’s t-test were used. Correlation between various variables was assessed using Pearson’s correlation equation.

**RESULT**

Totally 120 subjects were included who were age and sex match in the age group 30-55 years. In that 60 CHD subjects (45 males and 15 females) with average age of 42.93 ±3.39 years and 60 healthy controls (39 males and 21 females) with average age of 41.30±3.82 [Table 1]. The Mean level of Total Cholesterol, TG, LDL-C and hs-CRP are increased in patients with CHD compare with the controls, were as the mean levels of HDL-C and NO didn’t vary significantly between the groups [Table 2].

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 ($r = -0.035$).
Table 1: Demographic characteristics of the study subjects

| Parameters          | Controls (n=60) | CHD subjects (n=60) | P-Value |
|---------------------|-----------------|---------------------|---------|
| Mean age            | 41.8 ± 9.7      | 42.3 ± 10.5         | <0.0001 |
| Male Sex (%)        | 39 (65%)        | 45 (75%)            | -       |
| Female Sex (%)      | 21 (35%)        | 15 (25%)            | -       |
| BMI (kg/m²)         | 21.53 ± 1.71    | 24.47 ± 0.61        | <0.0001 |
| WC(cm)              | 90.51 ± 4.45    | 84.77 ± 3.26        | <0.0001 |
| HC (cm)             | 98.8 ± 5.23     | 99.54 ± 3.11        | <0.0001 |
| W/H ratio           | 0.90 ± 0.04     | 0.84 ± 0.02         | <0.0001 |
| Systolic Blood Pressure | 125.6±4.2      | 117.5±3.3           | <0.0001 |
| Diastolic Blood Pressure | 80±1.2         | 74.3±6.2            | <0.0001 |

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

Table 2: Biochemical parameters of Coronary Heart Disease Subject and normal individuals

| Parameters                  | Controls (n=60) | CHD Subjects (n=60) | P-Value (r-value) |
|-----------------------------|-----------------|---------------------|-------------------|
| FBG(mg/dl)                  | 93.86±6.46      | 99.3±11.54          | <0.0018           |
| Total cholesterol (mg/dl)   | 168.8±16.3      | 239±41.42           | <0.001            |
| Triglyceride (mg/dl)        | 84.6±30.5       | 159.7±69            | <0.001            |
| HDL (mg/dl)                 | 46±9            | 34±7                | <0.001            |
| LDL (mg/dl)                 | 106±12.59       | 161.9±27.46         | <0.001            |
| TC/HDL Ratio                | 3.71±0.70       | 6.17±1.14           | 0.0982            |
| LDL/HDL Ratio               | 2.35±0.53       | 4.22±0.75           | 0.1023            |
| hs-CRP (mg/L)               | 1.92±0.47       | 6.80±1.35           | <0.001            |
| Nitric Oxide (µmol/L)       | 19.08±0.74      | 12.97±1.20          | <0.001            |

FBG- Fasting Blood Glucose, TC- Total Cholesterol, TG- Triglyceride, HDL- High Density Lipoprotein, LDL- Low Density Lipoprotein, NO- Nitric Oxide, hs-CRP- High sensitive C - reactive protein; Values expressed as Mean±SD; *P-value < 0.05 is considered to be significant; NS-Not significant; ***Very Highly significant; **Highly Significant

Table 3: Pearson correlations analysis between Nitric Oxide and hs-CRP with other biochemical parameters in subjects with CHD

| NO          | hs-CRP      | P-Value |
|-------------|-------------|---------|
| BMI         | 0.160 a     | 0.141 a  | <0.0001 *** |
| Waist circumference | -0.035 b  | 0.065 a  | <0.0001 *** |
| Waist Hip Ratio          | -0.027 b  | 0.286 a  | <0.0001 *** |
| FBG          | -0.150 b    | -0.152 b | <0.0001 *** |
| Total Cholesterol       | -0.812 b   | -0.071 b | <0.0001 *** |
| Triglyceride            | 0.036 a    | 0.188 a  | <0.0001 *** |
| HDL-C                   | 0.046 a    | -0.015 b | <0.002 **   |
| LDL-C                   | -0.999 b   | 0.020 a  | <0.0001 *** |
| VLDL-C                  | -0.113 b   | 0.188 a  | <0.0001 *** |
| TC/HDL Ratio            | -0.059 b   | 0.541 a  | <0.0001 *** |
| LDL/HDL Ratio           | -0.066 b   | -0.023 b | <0.0001 *** |
| Nitric Oxide            | -         | -0.995 b | <0.0001 *** |
| hs-CRP                  | -0.995 b   | -       | <0.0001 *** |

a- Positive Correlation; b- Negative Correlation
*P-value < 0.05 is considered to be significant; NS-Not significant; ***Very Highly significant; **Highly Significant
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- 0.027), FBG (r = -0.150), Total Cholesterol (r = -0.812), LDL-C (r = -0.999), VLDL-C (r = -0.113), TC/HDL Ratio (r = -0.059), LDL/HDL Ratio (r = -0.066) and ox-LDL-C (r = -0.995) and hs-CRP were positively correlated with BMI (r = 0.141), Waist Circumference (r = 0.065), Waist Hip Ratio (r = 0.286), Triglyceride (r = 0.188), LDL-C (r = 0.020), VLDL-C (r = 0.188) and TC/HDL Ratio (r = 0.541). hs-CRP negatively correlated with FBG (r = -0.152), Total Cholesterol (r = -0.071), HDL-C (r = -0.015) and LDL/HDL Ratio (r = -0.023) [Table 3].

DISCUSSION

Nitric oxide is a free radical gas and acts as an inflammatory mediator due to its versatility in both pro and anti-inflammatory effects. Nitric oxide involved in numerous biological function comprising vasodilation, neurotransmission, inflammation and macrophage-mediated immunity (Sproston et al., 2018). There are more than a few factors that affect the efficiency of Nitric Oxide. Elevated level of LDL suggests the increased inflammatory events in patients who are positively correlated with the progression of CHD. 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). The events of CHD arise with the high LDL deposition in endothelial wall (Leiva et al., 2015) due to over production of reactive oxygen species (ROS) by endothelial cells, through the oxidative modifications leads to endothelial dysfunction and plaque disruption (Singh et al., 2002). Endothelial injury, which causes increased vascular permeability, leukocyte adhesion, and thrombosis (Reglero-Real et al., 2016). During the process of inflammation, pro-inflammatory cytokines associated with nitric oxide expression in monocytes and macrophages as well as in neutrophils produce enormous volumes than normal physiological concentrations (Sharma et al., 2007). During inflammation, an acute-phase protein released into the blood by liver accompanying with the development of coronary heart disease. Higher level of hs-CRP is accepted as a major risk aspect for the progression of coronary heart disease (Fonseca and Izar, 2016). Ridker et al., in his study stated that the healthy subjects with elevated hs-CRP values are 4 times possible to have coronary heart disease (Ridker et al., 1997). Ndrepepa et al. stated that raised hs-CRP level is interconnected with the threat of future cardiovascular actions (heart attack, stroke and death) in healthy subjects and also in subjects with coronary heart disease. Decreased level in hs-CRP and LDL cholesterol are associated with a fall level in progression of atherosclerosis and recover the risk of CHD (Ndrepepa et al., 2006). Inflammation promotes endothelial dysfunction and atherogenesis. It is a significant risk aspect in CHD. Sproston et al. stated that expression of adhesion molecules are up-regulated by CRP which are inhibited by endothelial nitric oxide synthase (eNOS) (Sproston and Ashworth, 2018). Jean Davignon et al. also reported that NO production repressed by CRP, which down regulates the eNOS in cardiovascular endothelial cells by obstructing angiogenesis which stimulate the pathogenesis of atherosclerotic vascular disease by vasoconstriction, leukocyte adherence and inflammation (Davignon, 2004).

CONCLUSION

Current study concludes that the subjects with low grade systemic inflammation and coronary heart disease is related with increased oxidative stress leads to impair nitric oxide bioavailability. Reduced NO, increased hs-CRP levels in patients may be a strong predictor of coronary heart disease.

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Conflict of Interest

The authors declare that there is no conflict of interest for this study.

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