



Correlation of serum Nitric Oxide and hs-CRP in non-smoker and non-diabetic patients with Coronary Artery Disease

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Abstract

Aim; The nitric oxide (NO) decrease and inflammation are cooperative events involved in atherosclerosis development. In the present study we assessed the correlation of NO with high sensitive c- reactive protein (hs-CRP) in the patients with coronary artery disease (CAD). Significant risk factors such as cigarette and diabetes were excluded from the study.

Materials and Methods; One hundred sixty subjects including 80 patients with angiographically diagnosed CAD and 80 age and sex matched CAD-free subjects as control were studied. The levels of NO in the samples were measured with the Griess method. Hs-CRP was measured by Commercial Kit (PARS AZMON.IRAN) by ImmunoTorbidity.

Results; Comparing with the control reduced levels of NO was noticed in the patient group ($p=0.02$) and the serum hs-CRP levels were increased significantly as compared to controls ($p=0.001$). In patient group was reverse correlation between NO with hs-CRP, but this correlation was not significant ($p=0.23$). In control group was direct correlation between NO with hs-CRP, but this correlation was not significant ($p=0.33$).

Conclusion; It is concluded that, in patients with stable coronary artery disease, low grade systemic inflammation is associated with increased in vivo oxidative stress leading to impaired systemic bioavailability of nitric oxide.

Key Words; Nitric Oxide; Hs-CRP; Non-smoker; Non-diabetic; Coronary Artery Disease.

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INTRODUCTION

Coronary Artery Disease (CAD) is one of the commonest causes of mortality and morbidity all over the world (1). The significance of risk factors such as diabetes mellitus (DM), hypertension, smoking, family history of premature heart disease, dyslipidaemia, male gender and advanced age has been instituted in predicting the risk of CAD by many studies. However, in half of those who expand CAD, the instituted risk factors are widely invisible (2). Recognition of additional factors is therefore essential to know more efficiently those at risk of CAD. Endothelial inflammatory proceedings have now been knowed in the pathogenesis of atherosclerosis (3).

Atherosclerosis and chronic risk factors for CAD are connected with ruined endothelium-dependent vasodilator function. Significantly, endothelial vasodilator dysfunction helps to CAD development and cardiovascular happening speeds (4).

In the circulatory system, nitric oxide (NO) is substantially main because it makes regular the tone of tightly layered cells called endothelial cells. If these endothelial cells become dysfunctional, they can cause spasm. One of the important subjects in CAD is the dominance of such dysfunctional endothelial coronary arteries, which secure blood to the heart muscle. When these arteries become collected person with CAD can experiment angina pectoris, a heart pain. Only in the last few years have scientists finded that the defect of NO in the coronary arteries endothelial cells might help to the endothelial dysfunction (5). L-Arginine is the substrate for the synthesis of NO that it is necessary for arrangement vascular tone and hemodynamic. As a precursor for synthesis of NO, L-Arginine disports basic function in nutrition and metabolism (5). NO is synthesized by the NO synthetase and transformed in to its more constant forms nitrite (NO₂) and nitrate (NO₃) in plasma. As well as regulating blood pressure through its basic affect for example; vasodilatation, it also has suppressory function in thrombocyte aggregation, leukocyte adhesion, smooth muscle cell proliferation and LDL oxidation. The decline in synthesis and bioavailability is an significant stage in the expansion of atherosclerosis (6).

The acute-phase reactant hs-CRP has been displayed to be a potent autonomic prophesier of future cardiovascular incidents. We hypothesized that regardless of CAD extension and tensity, serum hs-CRP levels are an index of atheromatous plaque activity and vulnerability (7).

The study designed to Nitric oxide, inflammatory factor in decrease this disease in non-smoker and non-diabetic patients suffering from CAD. We therefore prospectively tested whether basal and stimulated NO bioavailability correlates with hs-CRP serum levels in this patients.

MATERIALS AND METHODS

Subjects: We studied 80 CAD patients and 80 controls. Individules were selected to random. The CAD group included 40 females and, 40 males with a mean age of 58years, ranging from 40-79 years. They had various degrees of stenosis in one or more of the main branch of coronary artery documented by coronary angiography. Patients with diabetes mellitus, renal disease, chronic obstructive pulmonary disease, hepatitis and smoker were excluded from the study group. The controls included 40 females, 40 males with a mean age of 52 years, ranging from 38-74 years. The subjects proved to be healthy by health screening and had no obstructions in the coronary artery by angiography.

Blood sampling: Blood samples were collected in the morning by venipuncture after an overnight fast and were allowed to clot at room temperature for about 1 hour. Sera were separated from cells by centrifugation at 1500 x g for 10 min and kept at -80°C.

Measurement of serum NO: The levels of NO in serum were determined colorimetrically by Griess method (8). In this method, NO undergoes a series of reactions with several molecules present in biological fluids including O₂⁻, O₂ and NO₂. The final products of NO invivo are nitrite (NO₂⁻) and nitrate (NO₃⁻). The relative proportion of NO₂⁻ and NO₃⁻ is variable and cannot be predictated with certainty. Thus the best index of total NO production is the sum of both NO₂⁻ and NO₃⁻. The method used in this study provides an accurate and convenient measurement of nitrate/nitrite concentration in a simple two-step process. The first step is conversion of nitrate to nitrite utilizing nitrate reductase. The second step is the addition of Griess reagent which convert nitrite into deep purple azo compound. Colorimetric measurement of the absorbance due to this azo chromophore accurately determines nitrite concentration.

Measurement of serum hs-CRP: Hs-CRP was measured by Commercial Kit (PARS AZMON.IRAN) by Immuno Torbidometry (9).

Statistical Analysis: Data were analyzed using the SPSS for windows statistical package version 17 and Independent samplest test, Spearman,s Correlation Coefficients test. Statistical significance was set at 0.05. Data were expressed as mean±SD or n (%).

RESULTS

Table 1 indicates the general characteristics of the observed study population. NO differences were noticed between the mean values of age, sex and family history of CAD in patient and control groups. The percent of hypertensive subject in the patient groups was significantly higher than that of control group (p=0.04).

Comparing NO levels in the patient (including non-smoker and non-diabetic) and control groups, significantly low levels of NO were noticed in the patients group (p=0.02) (Table 2).



Hs-CRP was also measured in the patient group and comparing with control group significant elevation was noticed ($p=0.001$) (Table 2).

In patient group was reverse correlation between NO with hs-CRP, but this correlation was not significant (Table 3, figure 1).

In control group was direct correlation between NO with hs-CRP, but this correlation was not significant (Table 3, figure 2).

DISCUSSION

Nitric oxide (NO) acts a necessary pattern in regulating vascular tone and hemodynamic. NO excites endothelial reproduction and angiogenesis, thereby playing a main pattern in wound healing and microcirculation. As well as NO bridles the release of endothelin-1 (a vasoconstrictor) (10).

Besides being an endothelium derived vasodilator molecule, NO also has important physiological and pathological effects. It can be synthesized in most tissue and cells. Its most prominent roles in cardiovascular system are blood pressure regulation, inhibition of thrombocyte aggregation, leukocyte adhesion, smooth muscle cell proliferation, and LDL oxidation (11). The decreases in production and bioavailability are associated with events that accelerate development of atherosclerosis such as vasoconstriction, thrombocyte aggregation, migration of monocytes to the vascular wall, oxidized LDL and foam cell production. The main hypothesis of our study was that increased oxidative stress could reduce NO synthesis. Although patients with CAD had increased MDA levels, no significant correlations were determined between MDA and NO. NO levels were tended to be higher than those of the control group (11).

NO levels showed a significant relation with higher BMI and hypertension in coronary artery disease. It was suggested that adipose tissue contains NO synthetase enzyme, and is thus a potential NO source (11). In a study performed with healthy individuals at adolescent age, it was demonstrated that serum NO levels highly correlated with BMI and that NO levels were significantly higher in obese individuals. In our study, serum NO levels were found significantly lowers in patients. Scribner et al. (12) reported that, NO levels are significantly lower when compared to those without hypertension. Again in the same study, NO levels of the coronary artery patients without hypertension were similar to those of the control group (12). In this study, serum levels of NO in patient groups were meaning fully higher than those of control and our observations confirm data from previous studies.

Super Oxide Dismutase (SOD) is a key antioxidant enzyme located strategically between the endothelium and vascular smooth muscle cells in the compartment of the arterial wall where NO is capable of being inactivated by O_2^{0-} . Therefore, conditions resulting in NO depletion, such as atherosclerosis, could be associated with a concomitant decrease in extracellular SOD.

There is increasing evidence that inflammation acts a main role in athogenesis of atherosclerosis and its taxes. It has been offered. That hs-CRP may not only be a marker of commoned inflammation but also straightly and actively participate in atherogenesis (1). Increased hs-CRP concentration is associated with metabolic disorders such as dyslipidemia. Thus, increased CRP levels and dyslipidemia have significant risk prediction value compared with those based on lipids alone in CAD patients. Many studies have shown an increase in serum hs-CRP levels in CAD patients of more than 50 years of age in males and females (13). Present studies on CAD patients revealed a significant increase in the level of hs-CRP in these patients. The results are in agreement with those reported by Espliguero et al. This increase in CRP concentrations might be associated by the fact that CRP binds to the LDL particle in atherosclerotic plaques leading to activation of complement, thus, being proinflammatory and contributing to atherogenesis. CRP may also increases ischemic tissue damage by complement dependent mechanism and tissue factor production by macrophages.

The results of the present study demonstrate that the CRP serum level is an important independent predictor of both basal and stimulated NO bioavailability in the systemic circulation of patients with established CAD. Mechanistically, increased oxidative stress appears to be a major contributor to the reduced NO bioavailability associated with elevated CRP serum levels.

The present study is the first to document that basal systemic NO bioavailability correlates with hs-CRP serum levels in patients with established CAD. Where as a previous study reported an association between the effects of NO and hs-CRP serum levels in healthyvolunteers (14), the results of the present study demonstrate for the first time that endothelial activation, associated with elevated hs-CRP serum levels, is characterized by an impaired systemic bioavailability of NO in patients with CAD. The two fundamental mechanisms for impaired bioavailability of NO are reduced synthesis and increased oxidative inactivation by reactive oxygen species. Thus, enhanced oxygen-derived free radicals may, at least in part, contribute to the impaired systemic bioavailability of NO in patients with elevated hs-CRP serum levels.

CONCLUSION

It is concluded that, in patients with stable coronary artery disease, low grade systemic inflammation is associated with increased in vivo oxidative stress leading to impaired systemic bioavailability of NO, which might importantly contribute to the transition from stable CAD to acute coronary syndromes. Thus, improving NO bioavailability might represent a suitable target for therapy. Also these findings indicate that serum CRP levels are a marker of CAD activity and may be a biochemical marker of the diffuse inflammatory process that leads to multifocal plaque in stability. Thus, basal systemic NO bioavailability correlates with hs-CRP serum levels in patients with Coronary Artery Disease.



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LEGENDS

Table 1: The demographic and clinical data of the patient and control groups

Table 2: The levels of NO and hs-CRP in patient and control groups

Table 3: Correlation between NO with hs-CRP in patient and control groups

Figure 1: Scatter plot between hs-CRP and NO in patient group

Figure 2: Scatter plot between hs-CRP and NO in control group

Table 1: The demographic and clinical data of the patient and control groups

| Characteristics | Patients (n=70) mean±SD | Controls (n=70) mean±SD | Pvalue* |
|-----------------------|----------------------------|----------------------------|---------|
| Female/male,n | 35/35 | 35/35 | NS |
| Age,years | 57±8.82 | 56±10.06 | NS |
| Hypertension,n(%) | 43 (61%) | 32 (46%) | P=0.04 |
| Family history, n (%) | 34 (49%) | 28 (40%) | NS |

*Pvalue <0.05 considered significant.

Table 2: The levels of NO and hs-CRP in patient and control groups

| Parameter | Patients (n=70) mean ± SD | Controls (n=70) mean ± SD | P value * |
|---------------|------------------------------|------------------------------|-----------|
| NO(μM/L) | 108.68 ± 65 | 138.33 ± 70.98 | P=0.02 |
| Hs-CRP(mg/dl) | 4.61± 2.52 | 1.98 ± 0.94 | P=0.001 |

*Pvalue <0.05 considered significant.

Table 3: Correlation between NO with hs-CRP in patient and control groups

| Groups | Spearman's Correlation Coefficients | P value* |
|----------------|-------------------------------------|----------|
| Patient (n=70) | - 0.15 | 0.23 |
| Control (n=70) | + 0.12 | 0.33 |

* pvalue <0.05 considered significant.

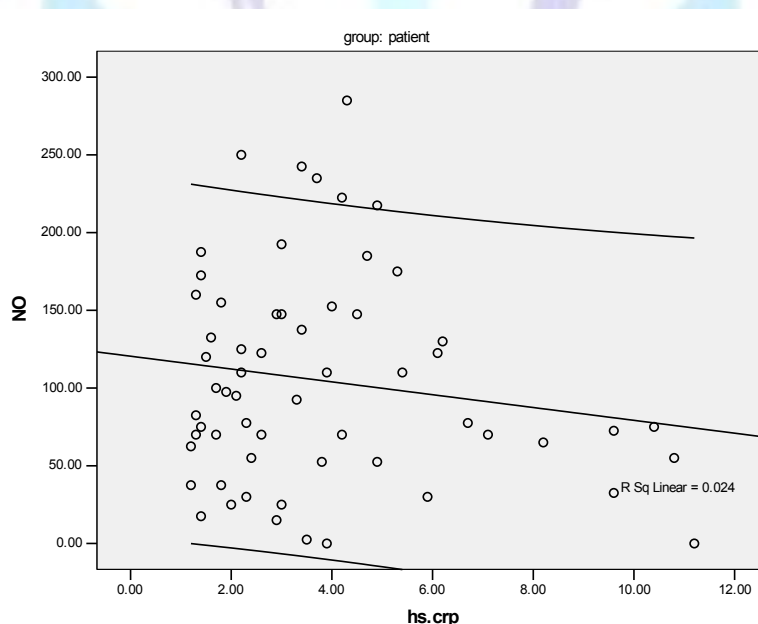


Figure 1: Scatter plot between hs-CRP and NO in patient group

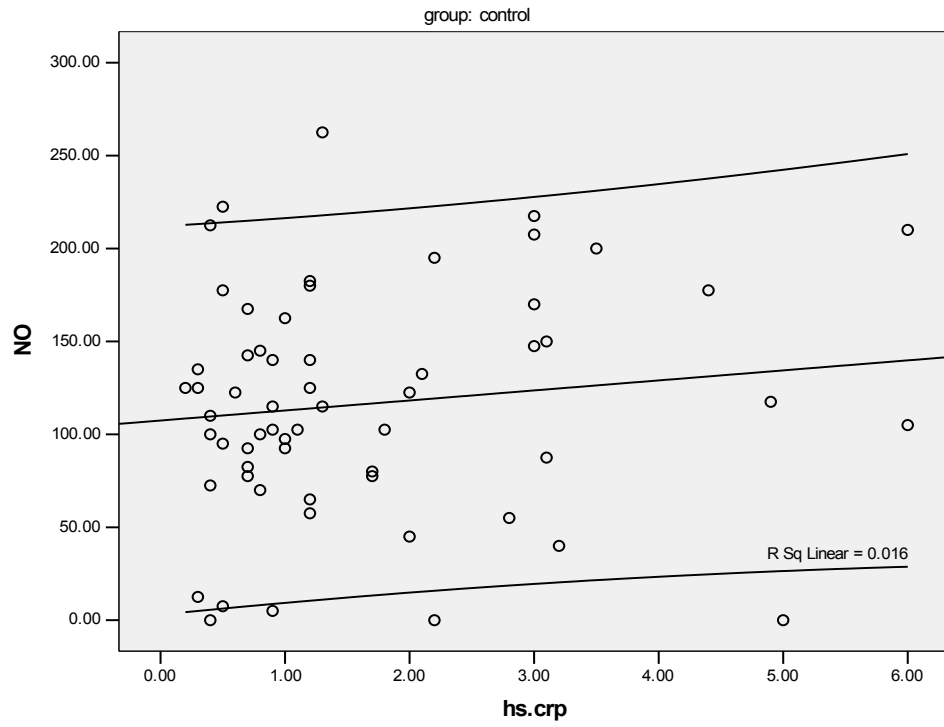


Figure 2: Scatter plot between hs-CRP and NO in control group