

# Plasma homocysteine and oxidative stress in cardiovascular disease

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**Abstract.** Hyperhomocysteinemia (Hhcy) has been associated with pathological and stressful conditions and is a risk factor for cardiovascular disease (CVD). The aim of this study was to evaluate the correlation between plasma homocysteine (hcy) and lipid peroxidation in patient with CVD. This study was carried out on 40 patients with CVD as well as 15 healthy volunteers of comparable age and gender as control group. The patients were divided into 2 groups as follows: group I, included 20 patients with acute myocardial infarction and group II, included 20 patients with atherosclerotic coronary artery disease with no evidence of previous myocardial infarction. Plasma hcy, nitric oxide (NO) and malondialdehyde (MDA) [as index of lipid peroxidation] were measured in all groups. In addition serum total-cholesterol, HDL, LDL and triglycerides were evaluated. Results obtained showed that, there was a significant elevation in the levels of plasma hcy, NO and MDA in groups I and II as compared to control group. There was a strong positive correlation between plasma hcy and MDA ( $r = 0.59, p < 0.001$ ). Also NO was positively correlated with both hcy ( $r = 0.49, p < 0.001$ ) and MDA ( $r = 0.51, p < 0.001$ ). Serum total cholesterol, LDL, and triglycerides were also significantly elevated while serum HDL was significantly decreased in groups I and II as compared to control group. It can be concluded that, hyperhomocysteinemia is a possible factor in free radical generation and therefore cardiovascular diseases.

## 1. Introduction

Oxidative stress occurs when there is an imbalance between free radical production and antioxidant capacity. This may be due to increased free radical formation in the body and/or loss of normal antioxidant defense, this disturbance has been associated with the development of cardiovascular disease (CVD) [29]. CVD and cerebrovascular disease is the leading cause of death [18]. Atherosclerosis of coronary arteries is responsible for almost all cases of CVD [12]. The identification of risk factors of atherosclerotic lesions leads to development of strategies for prevention of CVD and its complications. Conventional risk factors for coronary artery disease including hyperlipidemia, smoking, hypertension and diabetes are all associated with increased oxidative stress due to excess free radical activity in the vascular wall. This may facilitate the development of vascular disease [8].

Homocysteine (hcy) is a thiol containing amino acid produced by demethylation of methionine. Half of the hcy formed goes through the trans-sulphuration path-

way and the other half takes a methyl group from betaine or 5-methyltetrahydrofolic acid. Methionine synthesis is a cobalamin dependent enzyme which is functionally impaired when vitamin B12 metabolism is abnormal [32].

The nitric oxide (NO) is increasingly recognized as an important intra- and intercellular messenger [4]. The NO is identical to endothelium-derived relaxing factor and is the principle signal for relaxation of vascular smooth muscle cells [27]. The enzyme nitric oxide synthase converts L-arginine into citrulline and NO [22]. The role of NO has been implicated in a variety of biological processes such as neurotransmission, tumor cell killing, immunity, and inflammatory processes [15].

Experimental studies revealed that, infusion of hcy into animals results in damage of vascular endothelium and reduced platelet survival [16]. Hyperhomocysteinemia (Hhcy) has been associated with pathological and stressful conditions and is a risk factor for CVD [10]. This study was designed to evaluate the correlation among plasma hcy, lipid peroxidation indicator (MDA) and NO in patients with cardiovascular disease.

## 2. Subjects and methods

### 2.1. a) subjects

This study was carried out on 40 patients with CVD as well as 15 healthy volunteers of comparable age and gender as control group. The patients were divided into two groups as follows: Group I; included 20 patients (14 males and 6 females aged 42–60 years) with acute myocardial infarction and; group II; included 20 patients (12 males and 8 females aged 40–58 years) with coronary atherosclerosis with no evidence of previous myocardial infarction. The patients were diagnosed at the cardiology department, Ain Shams University Hospital. Patients with diabetes mellitus, renal impaired function were excluded from this study.

All subjects (patients and control) were subjected to clinical examination, echocardiographic imaging, electrocardiography, angiographically and treadmill exercise stress test. Diagnosis of myocardial infarction was based on the presence of two or more of the following criteria (i) typical anginal chest pain lasting for 30 minutes or more. (ii) presence of ST segment elevation in resting electrocardiography. (iii) elevation of the cardiac enzymes (creatinine kinase [CK] or CK,MB) twice or more the upper normal level. Meanwhile patients with coronary atherosclerosis were chosen from patients attending the cardiac catheterization laboratory for coronary angiography. The diagnosis was made when there was 50% or more luminal narrowing of one or more of the measure coronary arteries. Fasting venous blood samples were taken on the second day of attack, plasma and serum were separated and kept at  $-70^{\circ}\text{C}$  till analysis.

### 2.2. b) Methods

1. Plasma homocysteine was assayed by ELISA, as described in Frantzen et al. [13].
2. Plasma lipid peroxidation indicator, malondialdehyde (MDA) was evaluated by thiobarbituric acid according to Zima et al. [41].
3. Plasma nitrite and nitrate were assayed by the modified microassay as described by Vodovotz [36]. Briefly, nitrate in plasma was reduced to nitrite by Cadmium and assayed by Griess reagents.
4. Lipids profile was evaluated including, total cholesterol [17], HDL-cholesterol [24], LDL-cholesterol [14] and triglycerides [37].

Results are expressed as Mean  $\pm$  SD. The mean, standard deviation and p values were performed using the statistical package for the social science (Spss) whereas the correlation coefficient was performed using excell 5 on IBM, PC computer.

## 3. Results

Table 1, showed that, plasma levels of hcy, MDA and NO were significantly elevated in patients with acute myocardial infarction and coronary artery atherosclerosis ( $p < 0.001$ ,  $0.001$  and  $0.001$ ) as compared to control subjects. A statistically positive correlation was obtained between hcy and MDA ( $r = 0.59$ ,  $p < 0.001$ ). Also, plasma NO level revealed statistically significant positive correlation with plasma MDA ( $r = 0.59$ ,  $P < 0.001$ ) and plasma hcy ( $r = 0.5$ ,  $p < 0.001$ ). Lipid profile results revealed that, serum total cholesterol, LDL and triglycerides were significantly elevated while HDL was significantly decreased in groups I and II as compared to healthy subjects (the % changes were 44%, 54% for total cholesterol, 57.2%, 69% for LDL, 63%, 82.5% for triglycerides and  $-26.4\%$ ,  $-31\%$  for HDL respectively as compared to control group (Table 2).

## 4. Discussion

Traditional risk factors for coronary artery disease (CAD) such as hypertension, smoking and diabetes mellitus are all associated with increased oxidative stress due to excess free radical activity in the vascular wall. This may facilitate the development of vascular disease because of increased oxidation of LDL-particles which increases propensity to deposition in the vascular wall [25].

Epidemiological studies have consistently demonstrated that, high plasma hcy is an independent risk factor for atherosclerosis, as important as serum cholesterol level [9]. However, unlike hypercholesterolemia, hyperhomocysteinemia (Hhcy) is not a sufficient stimulus for the development of atherosclerosis but predisposes to complications and progression of the arterial lesion [3]. In addition, Hhcy is common in patients with peripheral arterial occlusive disease, coronary heart disease, cerebrovascular disease, carotid artery stenosis and venous thromboembolism [33]. This study aimed to evaluate the correlation between plasma hcy and oxidative stress in patients with cardiovascular diseases (CVD). The results obtained showed that there was a

Table 1  
Plasma levels of homocysteine (hcy), lipid peroxidation indicator (MDA) and nitric oxide (NO) in all studied groups (Mean  $\pm$  SD)

Groups	Plasma hcy umol/L	Plasma MDA MDA umol/L	Plasma NO umol/L
Control group (n = 15)		–	
Range -	(4.5–14.2)	(0.5–2.9)	(8.1–16.1)
Mean $\pm$ SD	8.69 $\pm$ 2.7	1.32 $\pm$ 0.7	11.46 $\pm$ 2.6
Group I Acute myocardial infarction (n = 20)			
Range	(10.1–27)	(1.2–5.9)	(13–22.1)
Mean $\pm$ SD	21.6 $\pm$ 4.3	2.42 $\pm$ 1.08	17.69 $\pm$ 2.57
P value	< 0.001	< 0.001	< 0.001
%change	148.5%	83.3%	54.3%
Group II Atherosclerosis (n = 20)			
Range	(9.3–22)	(1–4.1)	(10.5–19.1)
Mean $\pm$ SD	16.91 $\pm$ 3.3	1.98 $\pm$ 1.0	15.1 $\pm$ 2.46
P value	< 0.001	< 0.05	< 0.001
%change	94.4%	50%	32.45%

P value compared to control. % change between groups I & II and control.

Table 2  
Serum total cholesterol HDL-Cholesterol, LDL-Cholesterol and triglycerides in all studied groups (Mean  $\pm$  SD)

Groups	Total cholesterol mg/dl	HDL-Cholesterol mg/dl	LDL-Cholesterol mg/dl	Triglycerides mg/dl
Control group (n = 15)				
Range	(166–216)	(40.1–52.3)	(90–135)	(95.6–148.3)
Mean $\pm$ SD	191.5 $\pm$ 18.6	42.8 $\pm$ 3.2	106.6 $\pm$ 18.9	130.9 $\pm$ 17
Group I Acute myocardial infarction (n = 20)				
Range	(190–350)	(26–37)	(110–240)	(120–244)
Mean $\pm$ SD	277.2 $\pm$ 38	31.5 $\pm$ 3.3	167.6 $\pm$ 37.5	212 $\pm$ 65
P value	< 0.001	< 0.001	< 0.001	< 0.001
%change	44%	–26.4%	57.2%	63%
Group II Atherosclerosis (n = 20)				
Range	(199–353)	(25.2–34.3)	(115–260)	(160–268.)
Mean $\pm$ SD	295.21 $\pm$ 38	29.4 $\pm$ 1.9	180.9 $\pm$ 41	238 $\pm$ 91
P value	< 0.001	0.001	< 0.001	0.001
%change	54.1%	–31%	69.6%	82.5%

P value compared to control. % change between groups I & II and control.

highly significant elevation in the levels of plasma hcy, MDA and NO in patients with either acute myocardial infarction or coronary artery atherosclerosis as compared to healthy ones (The % changes were 148.5% and 94.4% for hcy, 83.3% and 50%, for MDA, 54.3% and 32.45% for NO respectively). The positive correlation between plasma hcy and lipid peroxidation in this study may suggest the possible role of hcy in the release of reactive oxygen species. Wei and Quast [38]

found that, there was an excessive glutamate excitotoxicity, leading to enhanced generation of hydroxylradicals via a NO-mediated mechanism and resulting in severe ischemic injury. Mc Murry et al. [26] reported that, thiobarbituric acid reactive substances are longer lived products of lipid peroxidation that are increased in oxidative stress in heart failure and CAD but are known to be non specific. Protective effects of NO have been reported during cerebral and myocardial is-

chemia. These effects of NO are most probably indirect effects of NO as a consequence of its vasodilatory activity to increase the blood flow, its capability to inhibit adhesion of lymphocytes, monocytes, and neutrophils to the endothelium (thereby decreasing local  $O_2^-$  generation), and its ability to inhibit platelets aggregation, thus decreasing capillary occlusions [23].

*In vitro* studies, hcy may acutely induce oxidative stress by autoxidation of sulfhydryl group of homocysteine to generate hydrogen peroxide [31], by decreasing intracellular antioxidant defense (glutathione and glutathione peroxidase) [34] and by direct cytotoxic effects (in cultured endothelial cells) [2]. Cavalca et al. [7] evaluated the possible role of hcy in inducing oxidative stress in CAD, they reported that plasma hcy and MDA concentrations were significantly higher in CVD patients than in control.

Zhang et al. [40] reported that the vasodilator effect of NO is attenuated in the presence of hcy, also the infusion of hcy with copper inhibits NO related vasodilatory responses by scavenging of NO. This may be one of the mechanisms by which Hhcy predisposes to CVD. The significant elevation of NO level in this study could be a protective mechanism against Hhcy to produce S-nitrosohomocysteine, which is less injurious than hcy [20]. *In vitro* study, NO decreases hcy by its conversion to the vasodilative and antioxidant compound S-nitrosohomocysteine [6].

Khan et al. [19] reported that, the significant elevation of NO might reflect the elaboration of the angio-pathic free radical peroxynitrite through the generation of superoxide anions and hydrogen peroxide. Hhcy has been associated with premature atherothrombotic vascular disease. The pathophysiological mechanisms linking Hhcy to vascular disease have been extensively studied by Van Guldener and Stehouwer [35], they suggested that, hcy limits the bioavailability of NO, increases oxidative stress, stimulates smooth cell proliferation and alters elastic wall properties.

Experimental studies by Kitiyakara et al. [21] revealed that, oxidative stress and Hhcy culminate in abnormal vascular and endothelial regulation, functional NO deficiency, vascular hypertrophy and atherosclerosis. Welch et al. [39] hypothesized that, hcy contributes to atherosclerosis by affecting cytokine induced production of NO by vascular smooth muscle cells. Also the authors found that, inducible nitric oxide synthase (iNOS) activity and iNOS protein level were increased significantly in the hcy treated cells as compared to control.

Hyperhomocysteinemia-induced atherosclerosis is probably due to various factors, including endothelial

cell injury, inability to sustain S-nitrosohomocysteine formation because of imbalance between production of NO by endothelial cells and hcy, smooth muscle proliferation and thromboembolism [28].

Lipoproteins abnormalities play a critical role in atherogenesis and CAD, such as elevated LDL, reduced HDL, increased triglycerides rich lipoproteins (VLDL and intermediate density lipoprotein iDL) and increased lipoprotein (a) [1].

Table 2 showed that, there were a highly significant elevation in the levels of serum total-cholesterol, LDL and triglycerides, while serum HDL was significantly decreased in groups I and II as compared to control group (The % changes were 44%, 54.5% for total cholesterol, 37.2%, 69% for LDL, 63%, 82.5% for triglycerides and -26.4%, -31% for HDL respectively)

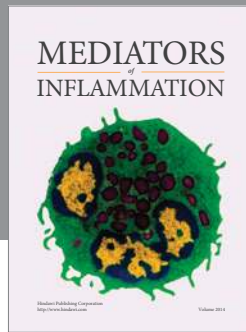
Study carried out by Dornner et al. [11] showed that, male patients with coronary heart disease had higher VLDL, triglycerides and low HDL than control while female patients did not have any significant difference in lipoproteins versus the controls.

Qujeg et al. [30] stated that, serum total hcy levels were significantly correlated with LDL-cholesterol ( $P < 0.05$ ,  $r = 0.98$ ) and HDL-cholesterol ( $P < 0.05$ ,  $r = 0.98$ ). Brouwer et al. [5] found that, male CAD patients had higher cholesterol, triglycerides, LDL-cholesterol, apo-B and decreased HDL-cholesterol and HDL/cholesterol concentration. Other risk factors were increased lipoprotein(a), Hhcy, renal disease and diabetes mellitus. We can conclude that plasma MDA, NO and hcy levels can be used as prognostic markers in patients with CVD.

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