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Relationship Between Hyperhomocysteinemia and Oxidative Stress with Severity of Atherosclerotic Lesion

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The aim of this study was to evaluate correlation between homocysteinemia and oxidative stress with severity of atherosclerotic lesion. We determined the concentration of total homocysteine in serum samples of subjects. The study group consisted of 88 selected patients with CAD, 52 male and 36 females, aged 39-85 (mean 48.65±3.81) years. The control group consisted of 39 normal volunteers, did not coronary artery disease, some sort of systemic screening, such as stress testing was done, 15 male and 24 females, age 38-73 (mean 42.73±0.79) years. The laboratory data of this group were used as a reference. The measurement of serum total homocysteine was performed using ELISA method. The mean serum total homocysteine level was 18.56±2.97 $\mu\text{M L}^{-1}$ for females and 17.34±2.26 $\mu\text{M L}^{-1}$ for males controls. The mean of total homocysteine level was 20.38±2.86 $\mu\text{M L}^{-1}$ for females patients and 22.25 ±2.44 $\mu\text{M L}^{-1}$ for males patients, ($p<0.05$). The mean serum total antioxidant level was 1.39±0.12 mM L^{-1} for females and 1.43±0.15 mM L^{-1} for males controls. The mean level of total antioxidant was 1.34±0.12 mM L^{-1} for females patients and 1.32±0.22 mM L^{-1} for males patients, ($p<0.05$). There were no statistically significant differences between levels of serum total homocysteine and total antioxidant in patients and control group. Also, there were no correlation between extension of atherosclerotic lesions and high serum total homocysteine. Results have shown that hyperhomocysteinemia has no important role in progress of atherosclerotic lesions.

Key words: Atherosclerotic, hyperhomocysteinemia, oxidative stress

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INTRODUCTION

The pathological accumulation of homocysteine in tissues and blood is generally considered to cause vascular complications by its injurious effect upon the endothelial cells^[1]. The etiology and clinical significance of hyperhomocysteinemia is under intense investigation. Although non-genetic and genetic factors influence plasma homocysteine concentrations, the etiology of hyperhomocysteinemia commonly found in-patients with coronary artery disease, cerebrovascular disease and peripheral vascular disease is often unclear^[2-5]. The concentration of homocysteine in plasma or serum is an established marker of common disease. It is a strong and independent risk factor for cardiovascular disease, a sensitive marker of cobalamin and folic acid deficiencies^[6-9]. Results from animal and cell culture studies indicate that increased homocysteine concentrations may accelerate coronary heart disease by various mechanisms, including direct damage to the vascular endothelium^[10], stimulation of smooth muscle cell proliferation^[11] and enhanced low density lipoprotein pre-oxidation^[12].

Induction of hyperhomocysteinemia in apoE-null mice by a diet enriched in methionine but depleted in folic acid and vitamins B6 and B12 increased atherosclerotic lesion area. These homocysteine-mediated effects were significantly suppressed, in parallel with decreased levels of plasma homocysteine-mediated, upon dietary supplementation with folic acid and vitamins B6 and B12^[13]. Although several cellular stress mechanisms have been proposed to explain the atherogenic effects of hyperhomocysteinemia, including oxidative stress, endoplasmic reticulum stress and inflammation, their association with atherogenesis has not been completely elucidated^[14]. Atherosclerosis is a slowly progressive process, involving the intima and media of large and medium sized arteries and leading to the formation of focal lesions, containing lipid and fibrous tissue. The measurement of metabolic markers of coronary risk (cholesterolemia, homocysteinemia and glycosylated hemoglobin) is useful to estimate the global coronary risk in the individual patient^[15]. Hyperhomocysteinemia, an important risk factor for cardiovascular disease, produces an endothelial damage due to oxidative stress; high plasma levels of homocysteine can be related either to genetic aberrations or to reduced blood concentrations of folic acid and vitamin B12^[16]. Homocysteinemia causes oxidative stress, decreases the aortic ability to generate prostacyclin and that antioxidants have a protective role^[17]. The occurrence of endothelial dysfunction could contribute to alterations of the endothelium-dependent vasomotor regulation. Elevated homocysteinemia

diminishes the vasodilation by nitric oxide, increases oxidative stress, stimulates the proliferation of vascular smooth muscle cells and alters the elastic properties of the vascular wall^[18]. Folic acid supplementation to hyperhomocysteinemic subjects resulted in a decrease in total blood homocysteine concentrations; moreover, there was a tendency to reverse the coagulation status and oxidative stress^[19]. Homocysteinemia is a major and independent risk factor for vascular disease. Oxidative stress is a possible mechanism for homocysteine induced vascular disease^[20]. The determination of serum total homocysteine and antioxidant level for the diagnosis of severity of atherosclerotic lesion patients will be an important feature of the clinical chemistry laboratory. The aim of this study was to evaluate correlation between hyperhomocysteinemia and oxidative stress with severity of atherosclerotic lesions.

MATERIALS AND METHODS

Reagents and chemicals: Homocysteine was purchased from Sigma Chemical Co. (St. Louis, Mo. and USA). All of the chemicals used were guaranteed-grade reagents and were used without further purification. All solutions were prepared with distilled deionised water.

Serum: Blood samples were obtained from blood donors at the Shahid Madani Hospital, Tabriz, Iran. It has been done in 2 years from March 2001 to January 2002. The population studied include 39 (controls) with no history of cardiovascular disease and 88 patients (cases).

Patient characteristic: The cases were mainly from the Shahid Madani Hospital of Tabriz, Iran. All patients had given prior informed consent for use of data and serum for research purpose. Their severity of atherosclerotic lesion status was confirmed by angiography. Patients scheduled for angiography at the Shahid Madani Hospital were eligible for inclusion in the study. After informed consent, patients were interviewed and a data collection form was completed that recorded clinical information. Each patient underwent venepuncture and collection of about 5 mL of blood for total homocysteine and antioxidant level. Severity of atherosclerotic lesions was analyzed according to the classification and grading angiography by a cardiologist. We determined the concentration of total homocysteine in serum samples of subjects. The study group consisted of 88 selected patients with CAD, 52 male and 36 females, aged 39-85 (mean 48.65±3.81) years. The diagnosis, based on criteria established by the World Health Organization, included typical or atypical chest pain, unequivocal changes in the

electrocardiogram and some sort of systemic screening, such as stress testing was done. The control group consisted of 39 normal volunteers, did not coronary artery disease, some sort of systemic screening, such as stress testing was done, 15 male and 24 females, age 38-73 (mean 42.73±5.79) years. The laboratory data of this group were used as a reference. Exclusion criteria were use of drugs affecting on serum homocysteine. The samples were obtained between 9 am and 1 pm, they were allowed to clot at room temperature for 1 to 4 h, were centrifuged at 2500 × g for 5 min and the serum was removed and stored without delay at -20°C until analysis of homocysteine. The measurement of serum total homocysteine was performed using ELISA method^[21]. Total antioxidant capacity of samples was determined by Autoanalyzer (COBAS MIRA plus model) using Randox kits. The correlation between the measured parameters and extension of atherosclerotic lesions were calculated using statistical analysis in the both groups.

Statistical analysis: Results are expressed as Mean±SD. Statistical significance between means checked by students t-test. p<0.05 was considered as level of significance.

RESULTS AND DISCUSSION

The mean serum total homocysteine level was 18.56±2.97 μM L⁻¹ for females and 17.34±2.26 μM L⁻¹ for males controls. The mean of total homocysteine level was 20.38±2.86 μM L⁻¹ for females patients and 22.25 ±2.44 μM L⁻¹ for males patients (Table 1). The mean serum total antioxidant level was 1.39±0.12 mM L⁻¹ for females and 1.43±0.15 mM L⁻¹ for males controls. The mean level of total antioxidant was 1.34±0.12 mM L⁻¹ for females patients and 1.32±0.22 mM L⁻¹ for males patients (Table 2). Figure 1 shows the relationship between the serum antioxidant level and serum total homocysteine concentration (r= -0.78).

The etiology and clinical significance of hyperhomocysteinemia is under intense investigation. Although non-genetic and genetic factors influence plasma homocysteine concentrations, the etiology of hyperhomocysteinemia commonly found in patients with coronary artery disease, cerebrovascular disease and peripheral vascular disease is often unclear^[2-5].

In the present investigation, we determined serum total homocysteine level in healthy men and women and evaluated relation of serum total homocysteine and serum total antioxidant level in the patients with coronary artery disease. However, in our study, we observed slight but statically insignificant increase of serum total

Table 1: Mean±SD of serum total homocysteine level in control subjects and patients with cardiac artery diseases

Gender group	Controls group (μM L ⁻¹)	Patients group (μM L ⁻¹)
Females	18.56±2.97	20.38±2.86
Males	17.34±2.26	22.25±2.44

Table 2: Mean±SD of serum total antioxidant status in control subjects and patients with cardiac artery diseases

Groups	Controls group (mM L ⁻¹)	Patients group (mM L ⁻¹)
Females	1.39±0.12	1.34±0.12
Males	1.43±0.15	1.32±0.22

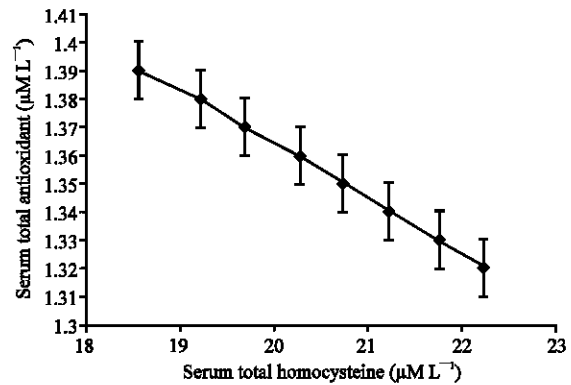


Fig. 1: Relationship between the serum antioxidant level and serum total homocysteine concentration. Each point is the mean of triplicate determinations with the standard deviation indicated by vertical bar (r= -0.78)

homocysteine in male patients. It may concluded that there were no statistically significant differences between levels of serum total homocysteine and total antioxidant in patients and control group. Also, there were negative correlation between extension of atherosclerotic lesions and high serum total homocysteine. We found that total homocysteine level in our subjects were somewhat higher than those reported in other populations^[2,4,9] which may be consistent with our subjects being selected for health. Part of this difference could be due, however, to the different methods used. Similarly to previous studies^[11,15,18], an important difference was observed between men and women. The current study demonstrates that serum total homocysteine level is higher in man than in women and that it increases significantly with age. Sex hormones may play a role for these sex and age differences. The sex difference has been ascribed to various factors, including different rates of homocysteine formation, the presence of a larger muscle mass and greater creatine phosphate synthesis in men and a lowering effect of estrogens in women. In the current study serum total homocysteine level

negatively correlated to antioxidant status in the controls and in patients with coronary artery disease. This observation is disagreement with the results obtained by other researchers^[5,12,17]. We have shown that hyperhomocysteinemia has no important role in progress of atherosclerotic lesions.

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