**Abstract:** Many risk factors for coronary heart disease (CHD) have been reported to date. Recently, it has been shown that elevations in homocysteine (Hcy) levels may contribute to the development of coronary heart diseases. In this study, our aim was to determine the relation of Hcy levels and other risk factors in CHD.

Serum Hcy, lipoprotein(a) [Lp(a)], C-reactive protein (CRP), fibrinogen, cholesterol, triglyceride, HDL and LDL levels were measured in 51 male patients, diagnosed with CHD with negative T elevations in electrocardiograms, and in 20 healthy males. Hcy was determined in serum samples with Fluorescence Polarization Immunoassay (FPIA) on Abbott IMx.

All the parameters were found to be significantly higher (p<0.05) when compared with those of the control group. The mean Hcy levels in patients and control subjects were 12.18±0.65µmol/L and 3.73±0.43µmol/L respectively. It was found that Hcy levels did not correlate with CRP, fibrinogen or Lp(a) (p>0.05), while CRP was well correlated with fibrinogen and Lp(a), and Lp(a) was well correlated with fibrinogen (p<0.05).

High levels of Hcy are associated with CHD independent of other coronary risk factors. Since high Hcy levels can easily be treated with vitamin supplements, determining Hcy concentrations on a routine basis may help to reduce mortality and morbidity from cardiovascular diseases.

**Key Words:** atherosclerosis, homocysteine, fibrinogen, CRP, Lp(a)

**Introduction**

Atherosclerosis, an important cause of mortality and morbidity, is a chronic inflammatory process which develops with the contribution of some risk factors. The atherosclerosis of coronary arteries is the cause of CHD. While age, sex and family history are unchangeable risk factors, hypercholesterolemia, hypertriglyceridemia, decreased HDL, hypercoagulable states, hypertension, diabetes, smoking, sedentary life style, obesity and personality are classic changeable risk factors in the pathogenesis of atherosclerosis (1).

It was first shown in 1969 that elevated levels of Hcy are associated with the development of atherosclerosis (2). Hcy is a sulfur-containing amino acid derived from the metabolism of methionine. Hcy seems to promote atherosclerosis by a variety of mechanisms. Experimental studies suggest that Hcy is probably the first agent that interferes with endothelial function, and foam cell generation comes as a later stage in the development of atherosclerosis (3).

Lp(a) is also identified as a risk factor in CHD. Many reports have suggested that increased levels of Lp(a) are correlated with CHD (4). The increased levels of CRP are correlated with the presence and severity of atherosclerosis and are strongly related to the occurrence of cardiovascular complications (5).

In this study, our aim was to show Hcy levels and their relation to the other risk factors in CHD.

**Materials and Methods**

Our study consisted of two groups of individuals. In group 1, there were 51 non-smoking male patients, mean age 54.76±1.66 years, admitted to the hospital with acute myocardial infarction (AMI). The diagnosis of AMI was based on typical chest pain, negative T elevations and raised cardiac enzyme levels. Group 2 was the control group and included 20 non-smoking males with a mean age of 46.42±2.70 years. This group had no clinical evidence of CHD.
Fasting blood samples for Hcy measurements were collected in 8mL Vacutainer tubes and kept on ice until centrifugation. For serum preparation, blood was centrifuged at 3000xg at 4°C for 10 minutes without delay and stored at —80°C until Hcy analysis.

For Hcy measurements, FPIA was used on an automatic analyzer (IMx, Abbott, Abbott Park, IL, USA). Lp(a) levels were determined by agarose gel electrophoresis (Sebia, Hyrys, France). CRP was measured immunoturbidimetrically on an automatic analyzer (Technicon RA-XT, Technicon Instruments, Tarrytown, N.Y. USA). For fibrinogen measurements, we used a coagulometric assay on an automatic analyzer (Option 4, bioMerieux, France). Cholesterol, triglyceride, HDL and LDL levels were determined using standard laboratory methods on an automatic analyzer (Olympus AU 5200, Olympus, Hamburg, Germany).

Statistical significance between the two groups was assessed by independent samples t-test and the correlation between tHcy, CRP, fibrinogen and Lp(a) within the patient group was evaluated by Pearson correlation coefficient. In evaluation of all the results, a p value <0.05 was considered significant. For these procedures, the Statistical Package for the Social Sciences (SPSS, version 9.0, SPSS Inc., Chicago, IL, USA) was used and the data are presented as mean±SEM.

### Results

All the risk factors for CHD were significantly different between the patients and control subjects in our study (p<0.05). The mean values for cholesterol, triglyceride, HDL, LDL, fibrinogen, Lp(a), CRP and Hcy are shown in Table 1. The correlation coefficients for CRP, Hcy, fibrinogen and Lp(a) are shown in Table 2.

The serum Hcy levels varied from 6.52 to 29.48 µmol/L (mean±SEM 12.18±0.65µmol/L) in 51 CHD patients and from 1.12 to 8.3 µmol/L (mean±SEM 3.73±0.43µmol/L) in 20 control subjects (Table 1). Of the 51 CHD patients, 8 (15.7% of total) had Hcy values greater than 15µmol/L.

When we examined the correlation of CRP, Hcy, fibrinogen and Lp(a) within the patient group, we found that fibrinogen showed a strong correlation with Lp(a)(r=0.796, p<0.05) and weak correlation with CRP levels (r=0.465, p<0.05). Lp(a) was also weakly correlated with CRP levels (r=0.412, p<0.05). We found no correlation between Hcy and the other risk factors in our study group.

### Discussion

Since atherosclerosis represents the underlying pathomechanism responsible for the majority of
myocardial infarction cases, it is considered to be the leading cause of death. Beside the established risk factors for CHD, the search for possible additional ones such as Lp(a), fibrinogen, CRP and Hcy is still in progress, because classic risk factors alone do not explain the high prevalence of this disease.

Studies in the last decade have indicated that hyperhomocysteinemia is an independent risk factor in the premature development of vascular diseases (6,7). Initial observations linking Hcy to vascular diseases were made by McCully more than 25 years ago in patients with homocystinuria, in whom total plasma Hcy can be as much as 50 times that found in normal subjects. However, recent studies have demonstrated that even mildly increased plasma Hcy can be a significant risk factor (8).

There are many prospective and retrospective studies about the association between hyperhomocysteinemia and CHD that show parallelism with our results. Stampfer et al. (9), Arnesen et al. (10), Wald et al. (11) and Nygard et al. (12) reported that Hcy concentrations were higher in patient groups with CHD than in control subjects. The mean concentration of Hcy varies between studies. Stampfer et al. studied 542 male participants and found Hcy mean concentrations of 11.1±4 versus 10.5±2.8µmol/L. Arnesen et al. performed a study in 601 participants and they also found Hcy concentrations higher in patient groups than with control subjects. The mean concentration of Hcy varies between studies. Stampfer et al. studied 542 male participants and found Hcy mean concentrations of 11.1±4 versus 10.5±2.8µmol/L. Arnesen et al. performed a study in 601 participants and they also found Hcy concentrations higher in the patients: 21.7±4.7 versus 11.3±3.7µmol/L.

In Wald et al.’s study, there were 229 patients who died of CHD. Their mean Hcy concentration was 13.1 versus 11.8µmol/L. Nygard et al.’s study included 587 patients and they also found higher Hcy concentrations in the patients. In our study, which included 51 patients that had survived myocardial infarction and 20 control subjects, mean Hcy concentrations were also higher than in the control subjects (12.18±0.65 versus 3.73±0.43µmol/L). The concentration of Hcy at which the risk begins to increase is not clear but all results show a strong association between elevated Hcy levels and CHD.

As there is a strong relation between cigarette smoking and Hcy levels, our study group consisted of non-smokers in order to eliminate the effect of smoking on tHcy levels. Multivitamin tablet usage is quite common in Turkey. In addition, fruits and vegetables, which are important sources of folic acid and pyridoxine, are produced and consumed in large quantities in Turkey. There is a high intake of foods of plant origin rather than animal protein consumption. Those facts may explain the lower tHcy mean levels in our study when compared to some other reports.

There was no correlation between Hcy and fibrinogen concentrations in our study (r=0.122, p>0.05). Malinow et al. (13) found a positive correlation between Hcy and fibrinogen. They assumed that high concentrations of Hcy may lead to the release of fibrinogen into plasma. As Hcy inhibits thrombomodulin and protein C activation, Hcy may lead to increased production of thrombin and fibrinogen and this may favor thrombosis. Increased fibrinogen levels are associated with acute coronary syndromes (5). Fibrinogen levels were significantly higher than those observed in the control group in our study (p<0.05). Fibrinogen had a weak correlation with CRP (r=0.465, p<0.05) and a strong correlation with Lp(a) (r=0.796, p<0.05).

The strong association between CRP levels and the risk of CHD has been shown in several studies (5,14). There is a positive association between CRP concentration and CHD risk. The increased levels of CRP and fibrinogen, both of them acute phase reactants, supports the idea that the inflammatory process has a role in these cases. The secretion of CRP is induced by cytokines, especially interleukin-6 (IL-6), and it has been suggested that IL-6 is the major mediator involved in the acute phase inflammation in AMI (15). CRP levels were significantly higher in our patient group than in the healthy control group (p<0.05). March et al. (16), Abdelmouttaleb et al. (5) and Delanghe et al. (17) found increased CRP concentrations in patients with AMI. Our results (4.70±0.34 versus 0.19±0.03mg/dL) confirm the existing relation between CRP levels and the presence of CHD. It was also proposed that CRP might play an atherogenic role through an interaction with LDL, but we found no correlation between CRP and LDL levels in our study group (r=0.03, p>0.05). This finding may suggest that CRP does not play a role as a promoter of chronic atherosclerosis.

Lp(a) is increased in patients with cardiovascular and cerebrovascular diseases. In 1963, Kare Berg first described Lp(a) as a lipoprotein antigen that was more prevalent in the plasma of myocardial infarction survivors (18). In this study, Lp(a) levels were higher when compared with the control group (p<0.05). Lp(a) showed a strong correlation with fibrinogen (r=0.796, p<0.05).
and a weak correlation with CRP levels (r=0.412, p<0.05). However, not all prospective studies have confirmed a positive relationship between Lp(a) and cardiovascular events. Some, such as the Lipid Research Clinic Study and the Gottingen Risk Incidence and Prevalence Study, have shown a positive relationship between Lp(a) and CAD, which is in accordance with our results. However, among others, the Helsinki Heart Study and the Physicians Heart Study have failed to show a relationship between plasma Lp(a) and CAD (19). The explanation of conflicting results may be due to the condition of the samples, the methodology used for assaying Lp(a), apo(a) isoform distribution, sample size and duration of follow up. We used agarose gel electrophoresis for the Lp(a) assay. This method has limited value because the minimal level of detection is 0.15-0.20g/L and this method does not give the exact Lp(a) levels. If we had used another method for Lp(a) determination, we could have obtained higher values in both groups but we do not think it would have made a great difference to the statistics.

Since Hcy is an independent risk factor in CHD, playing a significant role in the development of atherosclerosis, plasma Hcy concentrations should be determined, especially in patients who do not have other classic risk factors. This study does not show a causal relation between tHcy and mortality, but our results, like other ones, may suggest that determining Hcy concentrations on a routine basis may help to reduce mortality and morbidity from cardiovascular diseases since Hcy has been described as a common and easily reversible risk factor for occlusive atherosclerosis.

References