

# Relationship of Total Homocysteine, Cholesterol, Triglyceride in the Serum and Diastolic Blood Pressure of Patients with Myocardial Infarction

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## ABSTRACT

Recent studies have documented the importance of lipids and lipoproteins as determinants of serum total homocysteine concentrations in persons with myocardial infarction, and in healthy subjects. Associations between various biological systems and total homocysteine have also been reported. However, some of these associations are poorly understood. The purpose of our study was to measure serum total homocysteine concentrations in men and women who were selected for health and myocardial infarction to determine the relations of triglycerides, cholesterol and diastolic blood pressure. The study group consisted of 136 patients, 77 male and 59 females, aged 39-73 (mean 46.75  $\pm$  3.91). The control group consisted of 145 normal volunteers, 81 males and 64 females, aged 31-63 (mean 41.72  $\pm$  6.89). Serum total homocysteine was determined by gas chromatograph. Serum cholesterol and triglyceride were measured enzymatically on a spectrophotometer. Our results showed that serum total homocysteine concentrations were significantly positive correlated with cholesterol, triglyceride and diastolic blood pressure. *Iran. Biomed. J. 5 (2 & 3): 97-101, 2001*

**Keywords:** Homocysteine, Triglyceride, Cholesterol, Diastolic blood pressure

## INTRODUCTION

It is well-established that moderate hyperhomocysteinemia is an important cardiovascular risk factor [1, 2]. Homocysteine is toxic to vascular endothelium [3, 4]. It can potentiate the auto-oxidation of low-density lipoprotein cholesterol [5, 6] and can promote thrombosis [7, 8]. Hyper-homocysteinemia may be an additional risk factor predisposing individuals to premature coronary heart disease. Patients homozygous for cystathionine - $\beta$ -synthase deficiency, or those who have inherited disorders of cobalamin metabolism, have very high plasma homocysteine concentrations and are usually subjected to severe, premature atherosclerosis [9, 10]. The pathological accumulation of homocysteine in tissues and blood is generally considered to cause vascular complications by its injurious effect on the endothelial cells [11]. The etiology and clinical significance of hyperhomocysteinemia are under intense investigation. Although genetic and non-genetic factors influence on the plasma homocysteine concentrations, the etiology of

moderate to intermediate hyperhomocysteinemia commonly found in patients with coronary artery, cerebro-vascular and in peripheral vascular diseases [12-15]. The concentration of homocysteine in plasma or serum is an established marker of common disease. It is also a strong and independent risk factor for cardiovascular disease and a sensitive marker of cobalamin and folate deficiencies [16]. In this report, we investigate the relationship of total homocysteine, cholesterol, triglyceride in the serum and diastolic blood pressure of the patients with myocardial infarction.

## MATERIALS AND METHODS

**Reagents and chemicals.** Cation-exchange resin, (AG 50W-X8) and the anion-exchange resin (AG1-X8) were obtained from (Bio-Rad Laboratories, Richmond, CA). Homocysteine was purchased from Sigma Chemical Co. (St. Louis, Mo., USA). All of the chemicals used were guaranteed-grade reagents and were used without further purification. All

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solutions were prepared with distilled deionized water.

**Apparatus.** Dryness was performed on a vacuum concentrator (Savant Instruments, Inc., Hicksville, NY).

Sample analysis was performed on a MPC gas chromatograph with dual flame ionization detectors and with a Epson printer. Sample resolution was achieved on a fused silica capillary column (30 cm  $\times$  0.25 mm i.d., 0.25 mm film thickness). Spectrophotometer model was Cecil CE 1020.

**Serum.** Blood samples were obtained from subjects blood donors at the Shahid Beheshti Hospital. The study group consisted of 136 patients, 77 males and 59 females, aged 39-73 (mean  $46.75 \pm 3.91$ ). The control group with no history of cardiovascular disease consisted of 145 normal volunteers, 81 males and 64 females, aged 31-63 (mean  $41.72 \pm 6.89$ ). The diagnosis, based on criteria established by the World Health Organization, included typical or atypical chest pain and unequivocal changes in the electrocardiogram. Single myocardial infarction was subsequently confirmed by ECG criteria, and the appearance of pathologic Q wave accompanied by an elevation of the ST segment. Subsequently, inversion of the T wave together with significant elevations in serum glutamic oxaloacetic transaminase, creatine kinase and lactate dehydrogenase activity. The samples were obtained between 9 AM to 1 PM. They were allowed to clot at room temperature for 30 min. then, they were centrifuged immediately at  $2000 \times g$  for 5 min, and the serum was removed and stored at  $-20^{\circ}\text{C}$ .

**Sample preparation.** A volume of 100  $\mu\text{l}$  of  $\text{H}_2\text{O}$  containing 10  $\mu\text{mol}$  of homocysteine was added to a 150  $\mu\text{l}$  of serum. After mixing, 3.0 ml of  $\text{H}_2\text{O}$  containing 200  $\mu\text{g}$  of  $\text{Na}_2\text{EDTA}$  and 150  $\mu\text{l}$  of 2-mercaptoethanol were added followed by mixing and boiling at  $100^{\circ}\text{C}$  for 20 min. After cooling in room temperature, 200  $\mu\text{l}$  of  $\text{H}_2\text{O}$  containing 30.0 mg of sulfosalicylic acid, and 30.0  $\mu\text{l}$  of 6N HCl were added followed by mixing and centrifugation at  $1000 \times g$  for 20 min. The supernatant was then applied to disposable columns containing 300.0  $\mu\text{l}$  of the cation-exchange resin, AG 50W-X8 (200-400 mesh), hydrogen form that had been pre equilibrated with  $\text{H}_2\text{O}$ . After washing with 10 ml of  $\text{H}_2\text{O}$ , homocysteine was eluted with 3 ml of 8N  $\text{NH}_4\text{OH}$ . The eluates were applied directly to

disposable columns containing 300  $\mu\text{l}$  of the anion-exchange resin AG1-X8 (100-200 mesh), acetate form which had been washed and equilibrated with  $\text{H}_2\text{O}$ . After washing with 10 ml of  $\text{H}_2\text{O}$ , homocysteine was eluted in 3 ml of 0.2 M HCl and taken to dryness in a speed vacuum concentrator. The dried sample was then dissolved in 400  $\mu\text{l}$  of  $\text{H}_2\text{O}$ , transferred to 500  $\mu\text{l}$  Reacti-vials, and taken to dryness in the vacuum concentrator.

**Derivatization.** The t-butyl dimethylsilyl derivatives of homocysteine were prepared by adding 20  $\mu\text{l}$  of acetonitrile and 10  $\mu\text{l}$  of N-methyl-N (t-butyl dimethylsilyl) trifluoroacetamide to each vial. They were sealed with Teflon-lined septum caps, and allowed to stand at  $25^{\circ}\text{C}$ , overnight. Then, Hexane (200  $\mu\text{l}$ ) was added and after vortexing for 10 s, 30  $\mu\text{l}$   $\text{H}_2\text{O}$  was added. After vortexing for additional 10 s, the samples were centrifuged at  $2000 \times g$  for 5 min, then the upper hexane layer was decanted, transferred to microcentrifuge tubes, and dried to approximately 20  $\mu\text{l}$  by applying a stream of nitrogen. Sample (5  $\mu\text{l}$ ) was injected into the capillary column.

**Sample analysis.** The gas chromatograph was operated under standard autotune conditions with an injection port temperature of  $250^{\circ}\text{C}$  and a column head pressure of 70 kpa. Carrier gas was helium. The capillary column was equilibrated at  $250^{\circ}\text{C}$ . After 1 min sample injection, the temperature was increased to  $260^{\circ}\text{C}$  at  $5^{\circ}\text{C}/\text{min}$  and the interface temperature,  $5^{\circ}\text{C}$ . Data were collected from 0.0 to 7.0 min [17, 18].

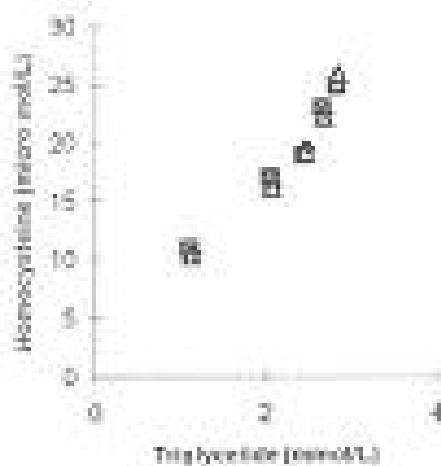
**Lipid measurements.** The concentrations of cholesterol and triglycerides were determined enzymatically with the cholesterol oxidase-peroxidase, 4-aminophenazone (CHOD-PAP) and glycerophosphate oxidase-peroxidase-4-aminophenazone (GPO-PAP) methods, respectively, on a Cecil CE spectrophotometer [19]. The interassay CV for determinations of total cholesterol and total tri-glycerides varied between 1.23% and 2.41% and between 1.87% and 3.46%, respectively.

**Statistical analysis.** The significance of differences between the mean of the control and test groups was determined by one-way analysis of variance followed by student's *t*-test.  $P < 0.05$  was considered statistically significant.

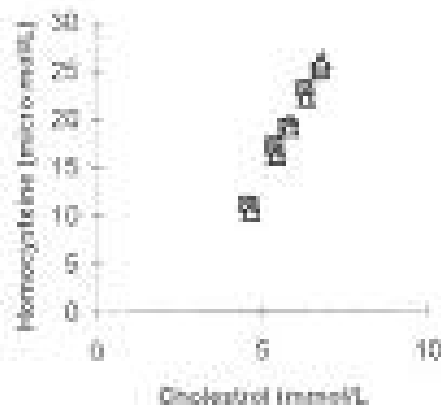
## RESULTS

The mean serum total homocysteine level for controls was  $10.55 \pm 1.48$   $\mu\text{mol/L}$  for females and  $12.75 \pm 1.26$   $\mu\text{mol/L}$  for males. The mean of total homocysteine was  $23.27 \pm 2.86$   $\mu\text{mol/L}$  for females case and  $25.06 \pm 2.44$   $\mu\text{mol/L}$  for male cases. The mean level was significantly higher in the patients with myocardial infarction than in the control ( $P < 0.05$ ). Serum triglyceride values were  $1.45 \pm 0.13$  and  $2.79 \pm 0.21$  mmol/l for men in the control and myocardial infarction groups, respectively. Healthy women had triglyceride levels of  $1.13 \pm 0.11$  vs.  $2.86 \pm 0.15$  mmol/l for women in the myocardial group. Total cholesterol levels were higher in patients than in controls,  $5.25 \pm 0.86$  vs.  $6.39 \pm 0.34$  mmol/l for control men and in myocardial infarction groups, respectively. The cholesterol levels in control women and in the myocardial infarction groups, were  $4.89 \pm 0.83$  vs.  $6.82 \pm 0.35$  mmol/l, respectively. Table 1 shows the mean serum total homocysteine, triglyceride, cholesterol and diastolic blood pressure levels in patients with myocardial infarction and in the control group.

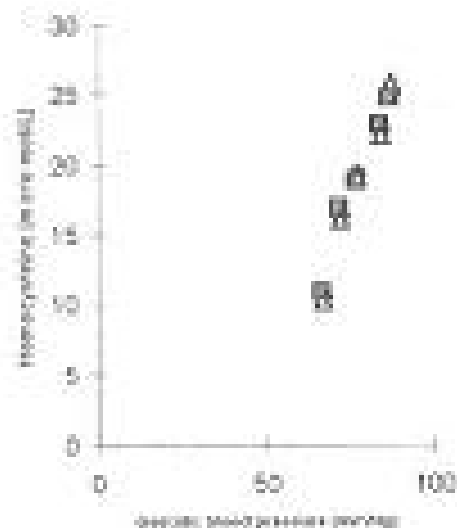
Figure 1 shows the relationship between the calculated means of serum triglyceride concentration and the serum total homocysteine concentration.



**Fig. 1.** Correlation of total homocysteine with serum triglycerides. Each point represents the mean value  $\pm$  SD of 3 separate experiments.  $Y = 2.29x - 0.74$ ,  $r = 0.86$ . Square = homocysteine.



**Fig. 2.** Correlation of total homocysteine with serum cholesterol. Each point represents the mean value  $\pm$  SD of 3 separate experiments.  $Y = 2.45x - 0.78$ ,  $r = 0.88$ . Square = homocysteine.



**Fig. 3.** Correlation of total homocysteine with diastolic blood pressure. Each point represents the mean value  $\pm$  SD of 3 separate experiments.  $Y = 2.35x - 0.73$ ,  $r = 0.85$ . Square = homocysteine.

The result showed that the serum triglyceride was significantly related with serum homocysteine. Figure 2 shows the relationship between the calculated means of serum cholesterol concentration and the serum total homocysteine concentration. The result showed that the serum cholesterol was significantly related to the serum homocysteine. Figure 3 shows the relationship between the calculated means of diastolic blood pressure level

**Table 1.** The mean serum total homocysteine, triglyceride, cholesterol and diastolic blood pressure levels in patient with myocardial infarction and control groups.

Biochemical parameter	Control group		Patient group	
	Male	Female	Male	Female
Homocysteine ( $\mu\text{mol/L}$ )	$12.75 \pm 1.26$	$10.55 \pm 1.48$	$25.06 \pm 2.44$	$23.27 \pm 2.86$
Triglyceride ( $\text{mmol/L}$ )	$1.45 \pm 0.13$	$1.13 \pm 0.11$	$2.79 \pm 0.21$	$2.86 \pm 0.15$
Cholesterol ( $\text{mmol/L}$ )	$5.25 \pm 0.86$	$4.89 \pm 0.83$	$6.82 \pm 0.35$	$6.39 \pm 0.34$
Diastolic blood pressure ( $\text{mm/Hg}$ )	$67.00 \pm 5$	$68.00 \pm 4$	$85.00 \pm 4$	$87.00 \pm 3$

values are mean  $\pm$  SD,  $P < 0.05$ .

and the serum total homocysteine concentration. The result showed that the diastolic blood pressure was significantly related to the serum homocysteine.

## DISCUSSION

The patients with hyperhomocysteinemia have higher risk factor profile than those without hyperhomocysteinemia. The increased risk in the patients with this abnormal finding was explained by the higher frequency of other risk factors. It is well known and established that important risk factors (elevated serum cholesterol levels, hypertension, tobacco consumption) can only explain 50% of coronary heart disease incidence [1, 2, 4]. The recent studies showed that 66% of patients with severe arteriosclerosis had no evidence of hypercholesterolemia, diabetes or hypertension. The measurement of circulating homocysteine levels together with the blood lipid profile are recommended in persons with atherosclerosis [3, 9, 10]. In this study, serum total homocysteine level was strongly associated with several biochemical variables that are known to be traditional harbingers of cardiovascular disease.

We determined serum total homocysteine concentrations in healthy men and women and evaluated the relation of serum total homocysteine, triglyceride and cholesterol with diastolic blood pressure in the patients with myocardial infarction. Also, we found that total homocysteine concentrations were somewhat higher in our subjects than those reported in other populations [12, 20] whom may be consistent with our subjects being selected for health. However, part of this difference could be due to the different methods used. Similarly to previous studies [21-23], an important difference was observed between men and women. The current study demonstrates that

serum total homocysteine level is higher in men than in women and it increases significantly with the age. Sex hormones may play a role for 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. Serum total homocysteine as a function of heart rate has not been investigated previously, whereas an association with blood pressure has been found only in hypertensive and diabetic patients. In the current study, serum total homocysteine level positively correlated to blood pressure in the controls and to both heart rate and blood pressure in patients with myocardial infarction. The mechanism behind the relation to blood pressure is unknown. In the present study, an association has consistently been observed between serum total homocysteine concentrations and serum triglyceride. This correlation may be explained by the fact that synthesis of homocysteine and synthesis of tri-glyceride are related. In addition to triglyceride, cholesterol concentration showed a strong association with total homocysteine in our subjects. In conclusion, an overwhelming amount of evidence supports the hypothesis that hyperhomocysteinemia is an important risk factor in myocardial infarction. It is too early to use serum homocysteine, like triglyceride and cholesterol for routine screening of healthy individuals.

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