Risk Factors in Ischemic Heart Disease: Comparison between Disturbance in Serum Lipid Profile and Total Homocysteine in Old Myocardial Infarction

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ABSTRACT

Objectives: *Disturbance in the Low-density lipoprotein cholesterol (LDL-C), High-density lipoprotein cholesterol (HDL-C), total cholesterol (TC), triglyceride (TG) and serum total homocysteine are predisposing factors in myocardial infarction.* ***Design and methods:*** *The study group consisted of 56 patients 35 male (aged 47.8±4.8 years), and 21 females (aged 46±4.3 years). The entry criterion for the patient group has a history of typical or atypical chest pain, unequivocal changes in the electrocardiogram. The control group consisted of 30 normal volunteers, 16 male (aged 48.4±5.2 years) and 14 females (aged 45.1±4.9 years). Measurement of serum total homocysteine was performed by enzyme linked immune sorbant assay (ELISA). Measurement of TC, TG, and HDL-C were performed using spectrophotometer. LDL-C was calculated.* ***Results:*** *Patients with myocardial infarction were found to have higher serum total homocysteine levels (23.93±2.99 μmol/L in male and 25.82±3.82 μmol/L in female) than controls (10.45±2.73 μmol/L and 12.92±0.9 μmol/L in both male and female respectively) (for each comparison; $p < 0.001$). Serum total homocysteine levels were significantly correlated with high Triglycerides and low HDL-C.* ***Conclusions:*** *The above mentioned findings suggest the potential usefulness of Triglycerides, HDL-C and serum total homocysteine as risk factors in myocardial infarction patients. These findings should be used in the future studies on the etiology and pathogenesis of myocardial infarction and to ascertain which patients are at risk for subsequent cardiovascular events and who will benefit from revascularization.*
Abbreviation*: LDL-C (Low density lipoprotein-cholesterol), HDL-C (High density lipoprotein- cholesterol), MI (Myocardial infarction), TG (Triglycerides) and Hcy (homocysteine)*

INTRODUCTION

Moderate hyperhomocysteinemia is an important cardiovascular risk factor[1,2]. Hcy is toxic to the vascular endothelium[3,4] and can potentiate the auto-oxidation of low-density lipoprotein cholesterol[5,6] and promotes thrombosis[7,8]. Hyperhomocysteinemia may be an additional risk factor predisposing individuals to premature coronary heart disease. Patients homozygous for cysta-

thionine-β-synthase deficiency, or who have inherited disorders of cobalamin metabolism, have very high plasma Hcy concentrations and are usually subject to severe premature atherosclerosis[9,10]. The pathological accumulation of Hcy in tissues and blood is generally considered to cause vascular complications by its injurious effect upon the endothelial cells[11]. The aetiology and clinical significance of hyperhomocysteinemia are under intense investigation. The aetiology of mild to moderate hyperhomo-cysteinemia commonly found in patients with coronary artery disease[12], cerebrovascular disease[13], peripheral vascular disease[14], and in patients with end-stage renal disease is often unclear[15,16]. Hcy is a strong and independent risk factor for cardiovascular disease, a sensitive marker of cobalamin and folate deficiencies[17,18]. Results from animal and cell culture studies indicate that increased Hcy concentrations may accelerate coronary heart disease by various mechanisms, including direct damage to the vascular endothelium[19], stimulation of smooth muscle cell proliferation[20], and enhanced LDL-C peroxidation[21]. Furthermore, Hcy may interfere with homeostasis by various mechanisms thus contributing to a biochemical environment induction of thrombus formation[22–24]. Hcy has, also, been shown to increase DNA synthesis in vascular smooth muscle cells consistent with early arteriosclerotic lesions and to induce these cells to proliferate while impeding the regeneration of endothelial cells and to cause oxidation of low-density lipoprotein[25-27]. The effects of Hcy on vascular hemostatic properties may be due to decrease in thrombomodulin cell surface expression and inhibition of protein C activation, thus probably contributing to development of thrombosis[28,29].

Different studies have used different Hcy levels to mark "abnormal" levels. However, the evidence is that Hcy is a graded risk factor, with the risk rising with even minor elevations of **Hcy. Bostom et al.**[30] find that serum Hcy >14 μmol/L considered a relative risk of vascular disease, but **Stehouwer**[31] considered that >17 μmol/L serum Hcy are highly risk of dying vascular disease.

Dyslipidemia is a well-established risk factor for cardiovascular disease[32]. In the Copenhagen Male Study (CMS), presence of a high fasting plasma TG concentration and a low high-density lipoprotein cholesterol (HDL-C) concentration, the character-istic dyslipidemia in the metabolic syndrome, was associated with a 2–foldhigher prevalence of cardiovascular disease (CVD) and a 2–fold-higher incidence of ischemic heart disease (IHD) in men without symptoms of CVD at baseline[33]. In addition, a link has been observed between a high TG level, low HDL-C level, reduced glucose tolerance, hyperinsulinemia, obesity, low physical activity, reduced fibrinolytic capacity and increased factor VII level[34].

The purpose of the present study was to compare serum total Hcy and TC, TG, LDL-C, and HDL-C as risk factors in old MI.

PATIENTS & METHODS

Clinical, ECG changes and Serum

The studied population were taken from Outpatient Clinic of the Coronary Diseases, Sohag University Hospital, Sohag Faculty of Medicine, Sohag University, in the period from July 2006 to February 2007. The study group consisted of 56 patients suffering from old MI above forty years old 35 male, and 21 females non pregnant women. The control group consisted of 30 normal volunteers, 16 male and 14 non pregnant females of the same age group. None of the subjects smoked cigarettes. Diagnosis was, based on criteria established by the World Health Organization, including typical or atypical chest pain, unequivocal changes in the electrocardiogram. Single MI was subsequently confirmed from ECG criteria, which was the appearance of pathologic Q wave accompanied by an elevation of the ST segment one mm or more in two or more contiguous leads, often with reciprocal ST depression in the contralateral and subsequently inversion of the T wave. Significant elevation in serum lactate dehydrogenase activity and creatine kinase MB level were associated in selected patients. Venous blood samples were obtained after the subjects had fasted for at least 12 hours and allowed to clot at room temperature for 30 min, were immediately centrifuged at 2000 rpm for 5 min, and the serum was removed and stored without delay at -20°C until analysis of Hcy and related lipids which was performed over a period of one h at most.

Homocysteine Assay

Axis Hcy enzyme linked immune sorbant assay (ELISA) is used for the determination of total Hcy in blood (Axis-Shield Diagnostics Ltd, United Kingdom)[35]. Protein-bound Hcy is reduced to free Hcy by use of dithiothreitol (DTT). Free Hcy converted enzymatically to S-adenosyl-L-homo-cysteine (SAH) by the use of SAH hydrolase and excess adenosine (Ad) in a separate procedure. The following solid-phase enzyme immunoassay is based on competition between SAH in the sample and immobilised SAH bound to the walls of the microtitre plate for binding sites on a monoclonal anti-SAH antibody. After removal of unbound anti-SAH antibody, a secondary rabbit anti-mouse antibody labelled with the enzyme horse radish peroxidase (HRP) is added. The peroxidase activity is measured at 450 nm after addition of substrate (N-methyl-2-pyrrolidine, propylene-glycol) and the absorbance is inversely related to the concentration of Hcy in the sample.

Cholesterol assay

Enzymatic colorimetric method was used for cholesterol assay from *Bicon-Diagnostk*[36]. This method depend on the presence of cholesterol esterase and cholesterol oxidase to yield cholesten-3-on and H_2O_2 (Hydrogen peroxide). Hydrogen peroxide created forms a red dyestuff by reacting with 4-aminophenazone and phenol under the catalytic action of peroxidase. The colour intensity is directly proportional to the concentration of cholesterol and can be determined photometrically at 546nm.

Triglycerides assay

Enzymatic colorimetric method was also, used for TG assay from *Bicon-Diagnostk*[37]. This method was based on the using of lipoprotein lipase from microorganisms for the rapid and complete hydrolysis of TG to glycerol followed by oxidation to dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide produced then reacts with 4-aminophenazone and 4-chlorphenol under the catalytic action of peroxidase to form a red dyestuff and measured at 546 nm.

HDL-Cholesterol assay

Chylomicrons, VLDL (very low density lipoproteions) and LDL-C were precipitated by phosphotungstic acid (PTA) and MgCl2 (ADWIC). After centrifugation the supernatant fluid contains the HDL-C fraction, then cholesterol content was determined enzymatically[38].

LDL-Cholesterol calculation

The LDL-C concentration was calculated from the difference between the total serum cholesterol, HDL-C and TG/5[39].

Statistical analysis

Values were expressed as mean, and ± standard deviation (SD). For serum lipids and Hcy in comparisons of controls and old MI patients, unpaired Student's *t*-test was used. Differences with p values <0.05 were considered significant by using SPSS software (release 10.0).

RESULTS

Fifty six patients (35 male and 21 female) suffering from old MI and 30 normal volunteers (16 male and 14 females) above forty were included in the study. The mean of age in old MI was 47.8±4.8 years in males and 46.±4.3 years in females. In the control group, the mean age was 48.4±5.2 years in males and 45.1±4.9 years in females. Patients with MI were found to have higher serum total Hcy levels than controls (P <0.01) (Fig. 2). The mean serum total Hcy level in control group was 11.6±2.41 µmol/L (10.45 ±2.73 µmol/L for males and 12.92±0.9 µmol/L for females), but that in old MI patients was 24.64±3.42 µmol/L (23.93±2.99 µmol/L for males patients and 25.82±3.82 µmol/L for females) (fig.1). Serum level of hemocysteine ranged in MI from 16.78 to 31.55 µmol/L, in control group ranged from 5.99 to 14.82 µmol/L. All MI patients had serum Hcy >14 µmol/L (100%) and only 55 of them had serum Hyc >17 µmol/L (98.2%). In control group all had serum Hcy below 17 µmol/L and 25 of them had serum Hyc > 14 µmol/L (83.3%). So, sensitivity and specificity of serum Hcy level > 14 µmol/L were 91.8% and 100% respectively. The sensitivity was higher (100%) with serum Hcy > 17 µmol/L.

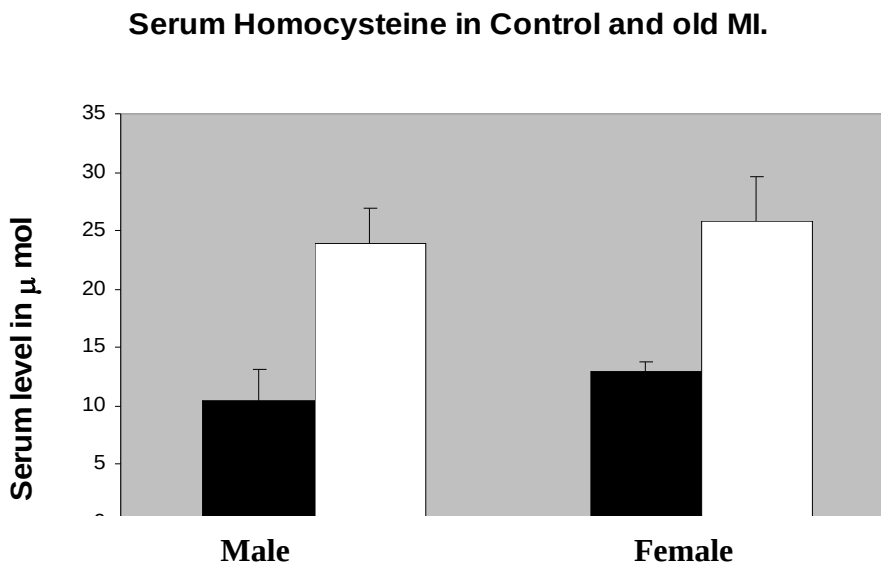

Fig.(1): Serum homocysteine in control (Black columns) and old MI (white columns) of male and female groups

Serum TG levels were higher in patients with old MI (320.5±10.7 mg/dL) than in controls (139.2±31 mg/dL) (P <0.05). The mean serum TG level was 192.38 ±12.37 mg/dL vs 343.19±34.79 mg/dL for men in the control and MI groups, respectively; and 150.41±27.57mg/dL vs 282.75±42.82 mg/dL for women in the control and MI groups, respectively, $P < 0.05$ in each groups (fig.2). 44 patients with MI had serum TG>200mg/dL (78.6%) but only 2 of control persons had hypertri-glyceridemia. (6,7%). So, Sensitivity and specificity of serum TG >200mg/dL were 95.8% and 73.7% respectively.

HDL-cholesterol levels were lower in patients with old MI (50.01±9.59 mg/dL) than in controls (57.53±10.18 mg/dL) (P <0.05). Its mean was lower in men and women with old MI (48.63±11.1 mg/dL and 52.33±5.83 mg/dL respectively, P, 0.05) than in healthy men and women (55.25±7.58 mg/dL and 60.14±12.3 mg/dL respectively) (fig.2). Only 3 patients with MI had serum HDL-C <35mg/dL (5.4%) but all the control persons had serum HDL-C above this level. The sensitivity and specificity of serum HDL-C <35mg/dL were 100% and 36.6% respectively.

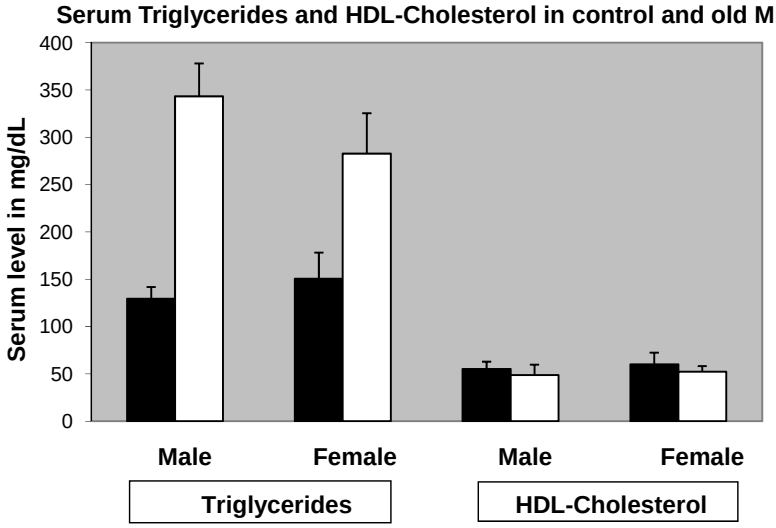

Fig.(2): Serum TG (first four columns) and HDL-C (following four columns) in both control (Black columns) and old MI (white columns) of male and female groups

DISCUSSION

Although the mortality following acute MI has decreased, morbidity remains considerable. The task of physician is to initiate risk factor modification, lipid-lowering therapy, smoking cessation and other intervention. Post-MI risk factors evaluation is used to ascertain which patients are at risk for subsequent cardiovascular events and who will benefit from revascularization. All patients who have had an MI should undertake aggressive modification of their risk factors. As seen in the present work, a relationship between serum total Hcy and/or one of two conventional risk factors for MI (TG and HDL-C) was observed. Results obtained in the present study, like those of other authors[2,21]; suggest that the serum total Hcy assay could be used to predict future risk for MI. There is no relation between serum total Hcy and LDL-C in these patients. Although hyperhomo-cysteinemia can potentiate the oxidation of low-density lipoprotein cholesterol[25-27] that can promotes thrombosis[7,8] but it has no apparent correlation effect on serum LDL-C. The average maximal value of all patients was at least two times greater than the normal subjects and the more sensitivity and specificity of serum Hcy (specially >17µmol/L) explain the high diagnostic value of the Hcy assay for MI. The results presented in the current work indicate that an elevated total Hcy level with or without high TG or low HDL-C are

risk factors in IHD but their relations to prognosis and severity of MI need further work. The current results were in good agreement with those reported previously[19-22]. The determination of serum total Hcy, TG and HDL-C for the diagnosis of MI patients will be an important feature of the clinical chemistry laboratory. Further work on the mode of action of Hcy in relation to MI is needed.

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عوامل الخطر في حالات قصور الشرايين التاجية
دراسة مقارنة بين الخلل في نسبة الدهون بالدم و الهوموستايين الكلى في مرض احتشاء عضلة القلب

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إن الخلل في معدلات نسب الدهون وارتفاع نسبة الهوموستايين بالدم من أهم العوامل المساعدة على حدوث مرض احتشاء عضلة القلب والقصور في الشرايين التاجية لذا فقد قمنا بعمل مقارنة بين هذين العاملين لمعرفة ماهي أدق العوامل الدالة علي توقع حدوث مثل هذه الأمراض وذلك بعمل دراسة علي ٥٦ مريضا قد تم حجزهم في العناية المركزة بمستشفي كلية الطب جامعة سوهاج بعد تشخيصهم بالوسائل اللازمة للتأكد من حدوث احتشاء في عضلة القلب ومقارنتهم بعدد ٣٠ فردا ليست لديهم أي إصابات بقصور في الشرايين التاجية وذلك من خلال قياس نسبة الهيموستايين , الكولسترول الكلي (TC)، الجلسريدات الثلاثية (TG), البروتينات الدهنية ذات الكثافة العالية (HDL-C), وآخري ذات الكثافة المنخفضة (LDL-C) وقد وجد أن من أهم العوامل الدالة علي خطورة التعرض لمرض احتشاء عضلة القلب تزداد بارتفاع نسبة الجلسريدات الثلاثية وانخفاض في نسبة البروتينات الدهنية ذات الكثافة العالية وأيضا الارتفاع المتوسط في نسبة الهوموستايين بالدم الذي يعتبر من أكثر العوامل الدالة علي ارتفاع نسبة حدوث الاحتشاء بعضلة القلب مقارنة بالعوامل الاخري من حيث أنها الأكثر شيوعا في هذه الحالات المرضية والأكثر حساسية وبذلك يمكن قياسه في الأشخاص فوق سن الأربعين وأيضا الأكثر عرضة لاحتشاء عضلة القلب.