

Full Length Research Paper

Association of plasma protein C levels and coronary artery disease in men

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Several studies have shown the risk factor causes of coronary heart disease. In this study we tested the hypothesis that plasma protein C level might be used as a biomarker for coronary heart disease and myocardial infarction. The study included 60 men that were classified into 3 groups according to clinical examination; group I set as healthy control group, group II set as patients with ischemic heart disease and group III set as patients suffering from myocardial infarction. Different parameters were measured including, coagulation factor prothrombin time, partial thromboplastin time, fibrinogen and protein C. The activity of the cardiac enzymes (creatine phosphokinase, creatine phosphokinase-MB and lactate dehydrogenase) was also measured. Finally, lipids profile (total lipids, phospholipids, triacylglycerol, total cholesterol, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein (HDL-C) were measured. The results demonstrate significant decrease level of protein C and prothrombin concentration (%) in ischemic heart disease and in myocardial infarction (MI) groups, when compared to the control group. Meanwhile, MI group showed more significant decrease comparing to IHD. Plasma protein C might serve as a marker for coronary artery disease in men. Further studies are warranted to bolster the data and to identify pathogenesis links between innate immune system activation and atherosclerosis.

Key words: Ischemic heart disease, myocardial infarction, protein C, coagulation factor, lipids profile.

INTRODUCTION

Atherosclerosis is a condition in which there is an artery wall thickness as a result of the accumulation of fatty materials such as cholesterol. It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, caused largely by the accumulation of macrophage white blood cells and promoted by low density lipoprotein (plasma proteins that carry cholesterol and triglycerides) without adequate removal of fats and cholesterol from the macrophages by functional high density lipoprotein (HDL). It is commonly referred to as a

hardening of the arteries. It is caused by the formation of multiple plaques within the arteries (Finn et al., 2010).

Myocardial infarction (MI) commonly known as a heart attack, results from the interruption of blood supply to a part of the heart, causing heart cells to die. This is most commonly due to occlusion of a coronary artery following the rupture of a vulnerable atherosclerotic plaque in the wall of an artery (Didangelos et al., 2009). Moreover, may be a minor event in a lifelong chronic disease, it may even go undetected, but it may also be a major

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Abbreviations: HDL, High density lipoprotein; MI, myocardial infarction; IHD, ischemic heart disease; O-LDL, oxidized low density lipoprotein; ECG, electrocardiogram; CPK, creatine phosphokinase; CPK-MB, creatine phosphokinase MB; APC, activated protein C; CAD, coronary artery disease; CVD, cardiovascular disease.

catastrophic event leading to sudden death or severe hemodynamic deterioration. Myocardial infarction may be the first manifestation of coronary artery disease, or it may occur, repeatedly, in patients with established disease (Thygesen et al., 2007). Disturbed lipid profile is one of the most important and potent risk factors in ischemic heart disease (IHD). It has been demonstrated that raised oxidative stress promotes several undesirable pathways including the formation of oxidized low density lipoprotein (O-LDL) and oxidized cholesterol which encourages cholesterol accumulation in arterial tissues (Maharjan et al., 2008)

Excessive consumption of saturated fat and cholesterol has been linked with increased concentration of plasma fibrinogen, a major risk factor for thrombosis that leads to heart attacks and strokes (Avogaro et al., 1988). Abnormalities in the blood coagulation factors regulating thrombosis may also contribute to the risk and extent of thrombosis (Hamker et al., 1991).

Homeostasis is a complex physiologic process involving a promoting factor (procoagulants) counter-balance by naturally occurring inhibitors. Derangement of this balance is considered to play an important role in the pathogenesis of thrombosis (Kenneth, 1992). Recently, it was found that heparin coagulation factor (HCII) inhibits thrombin activity by binding to derma tan sulfate and has been shown to be a novel and independent risk factor for atherosclerosis (Huang et al., 2008).

Human protein C is a vitamin K-dependent glycoprotein structurally similar to other vitamin K-dependent proteins affecting blood clotting, such as prothrombin, factor VII, factor IX and factor X. Protein C, also known as autoprothrombin IIA and blood coagulation factor XIV, is a zymogenic (inactive) protein, the activated form of which plays an important role in regulating blood clotting, inflammation, cell death and maintaining the permeability of blood vessel walls in humans and other animals (Mosnier et al., 2007).

In addition, activated protein C accelerates fibrinolytic activity by raising the level of plasminogen activator or by decreasing the level of plasminogen activator inhibitor (Zateishchikov et al., 1990). The determination of protein C makes it possible to identify patients who are at risk of thrombosis so that preventive measure can be instituted (Sturk et al., 1987). We tested the hypothesis that plasma protein C and different coagulation factors might be able to be used as a biomarker for coronary artery disease (CAD).

MATERIALS AND METHODS

Subjects

Sixty male subjects were selected from Health Insurance Hospital, Al-Azhar University, Cairo Egypt. Their ages ranged between 40-65 years old. They were classified into three groups after complete history taking and through clinical examinations and full investigations by electrocardiogram (ECG), echo, chest X-ray, and laboratory

examinations. Group I: Control group, twenty normal healthy men with no history for disease and drug intake. Group II: Twenty cases were suffering from ischemic heart diseases (IHD). Group III: Twenty cases were diagnosed as having MI disease. None of the study participants had any of the following disorders, associated with an acute phase reaction, febrile acute infection or acute state of a chronic infection or an inflammatory disease, underlying hematologic or malignant diseases and renal disorders. Current medication and sociodemographic characteristics were also recorded. Participation was voluntary, written informed consent was obtained from each subject upon entry into the study. The study was approved by the ethics committee of the University of Al-Azhar.

Blood sampling

Ten ml of blood were collected from each subjects, 5 ml blood were added to 3.8% trisodium citrate solution, in the proportion of 9 volumes of blood to one volume of anticoagulant solution and centerifugated at 3000 rpm for 10 min. The plasma was separated for determination of the following parameters: Activated prothrombin time (PT.second) according to Loeliyer et al. (1985). Activated partial thromboplastin time (PTT.second) was measured according to Munteam et al. (1992). Also plasma fibrinogen g/L and activated protein C% were measured according to Exner and Voasoki (1983).

The other 5 ml of blood were let to clot and the serum was used to determine the following parameters, activity of the enzyme creatine phosphokinase (CPK) according to the study of Szasz (1976) and the activity of the isoenzyme creatine phosphokinase MB (CPK-MB) (Szasz, 1976).

Also the activity of the enzyme lactate dehydrogenase (LDH) (Anon, 1977) was measured. Serum total lipid, phospholipids, triacylglycerol, cholesterol and HDL-C were also measured according to Knight et al. (1972), Henry (1974), Stavropoulos (1974) and Abell et al. (1952). Low density lipoprotein cholesterol (LDL-C) was measured according to the equation:

$$\text{LDL-C} = \text{total cholesterol} - \text{HDL-C} - \text{TG}/5$$

Statistical analysis

All results were expressed as mean \pm S.E of the mean. Statistical Package for the Social Sciences (SPSS) program, version 11.0 (Chicago, IL, USA) was used to compare significance among three groups. Difference was considered significant when $p \leq 0.05$.

RESULTS

It is clear from Table 1 that there was a highly significant increase in the level of prothrombin time (in seconds) in group II and III when compared to the control group. Also, there was a highly significant decrease in prothrombin concentration percentage in IHD and MI groups compared to the control group.

Moreover, MI group showed a significant decrease compared to IHD group. It could be concluded from this Table that partial thromboplastin time level showed highly significant increase in MI group as compared to IHD group. Plasma fibrinogen increased significantly in IHD and MI groups compared to the control group. Furthermore, IHD group showed lower value of plasma fibrinogen as compared to MI group. Finally, there was a highly

Table 1. Clinical Characteristics and Coagulation Factors in Control, IHD and MI groups.

Parameter	Control group (n=20)	IHD (n=20)	MI (n=20)
Age (yrs)	50.0 ± 11	60 ± 9.5*	56.0 ± 10.0
BMI (Kg/m ²)	27.0 ± 3.5	27.8 ± 3.4	27 ± 3.5
Current smoker, n (%)	6 (30.0)	15(75.0)	13 (65.0)
Prothrombin time (seconds)	12.4 ± 0.33	14.94 ± 0.84*	16.17 ± 1.09*§
Prothrombin conc.%	91.88 ± 6.13	57.6 ± 8.41*	49.7 ± 7.3*§
Partial thromboplastin (seconds)	33.74 ± 2.97	38.64 ± 6.99*	50.47 ± 6.24*§
Plasma fibrinogen (mg %)	262.8 ± 14.04	306.55 ± 7.33*	346.45 ± 16.63*§
Activity of Protein C (%)	109.53 ± 5.07	81.9 ± 7.77*	62.35 ± 5.13*§

Data are presented as mean ± SD, *p <0.05 vs. control, § p <0.05 significant (IHD) vs. (MI).

Table 2. The activity of cardiac enzymes in control, IHD and MI groups.

Parameter	Control group (n=20)	IHD (n=20)	MI (n=20)
Creatine phosphokinase (U/L)	90.15 ± 19.98	201.8 ± 13.82*	679.9 ± 17.807*§
Creatine phosphokinase –MB (U/L)	10.72 ± 2.62	35.45 ± 6*	107.25 ± 5.487*§
Lactate dehydrogenase (U/L)	114.1 ± 2.81	133.05 ± 3.047*	361.6 ± 10.473*§

Data are presented as mean ± SD, *p <0.05 vs. control, § p <0.05 significant (IHD) vs. (MI).

significant decrease in the levels of protein C in IHD and MI group as compared to control group. Meanwhile, MI group showed significant decrease in protein C level compared to IHD.

It could be seen from Table 2 that there was a highly significant increase in level of CPK, CPK-MB, and lactate dehydrogenase concentration in MI group as compared to IHD group. Also both IHD, and MI groups showed a highly significant increase compared to the control group. The results illustrated in Table 3 showed that lipid profiles (total lipids, total cholesterol, LDL-C) in MI and IHD groups were highly significant increased as compared to the control group, while HDL-C decreased significantly in these groups.

MI groups showed a significant increase in lipids profile as compared to IHD. The present study showed no significant change in phospholipids concentration in IHD group while there was highly significant increase in MI group as compared to the control group.

DISCUSSION

Prothrombin time (PT) measured the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) and indicates the overall efficiency of the extrinsic clotting system. The test depends on reactions with factors V, VII, and X and on the fibrinogen concentration of plasma (Dacie and Lewis, 1991). The results of this study are in agreement with that of Gupta et al. (1997) and Folsom et al. (1997). Also, our

results are in accordance with the study of Erbay et al (2004). The elevation of fibrinogen and prothrombin levels acts as a risk factor and may play a causative role in cardiovascular disease. Also, Chambliss et al. (1992) reported highly activated partial thromboplastin time and plasma factor VIII but decrease in value of protein C and antithrombin III activity. Also Kenneth et al. (1992) indicated that plasma fibrinogen concentration factor VII, protein C and antithromboplastin III levels were significantly higher in early atherosclerosis in carotid arteries which may be a useful marker for identifying individuals at high risk of developing arterial disorders.

In addition, this work showed a highly significant decrease in plasma protein C levels in IHD and MI group as compared to the control group. These results are in agreement with that obtained by Lauribe et al. (1992). The raised fibrinogen and decreased protein C appeared to be risk factor for sudden cardiac death. Gibbs et al. (1992) reported increase in the procoagulants fibrinogen, factor VIII, and decrease in protein C and antithrombin III in cases of myocardial infarction. Treatment with activated protein C significantly improved hemodynamic after ischemia-reperfusion and reduced ischemia-reperfusion-induced myocardial apoptosis in rats (Pirat et al., 2007). Henkens et al. (1993) observed that thromboembolic events occurred in 30% of protein C deficient and in 35% of protein S deficient persons. Also, Dahl back et al. (1993) reported poor anticoagulant response to activated protein C in several families with hereditary tendency to venous thrombosis. Moreover, It was also reported that concentration of protein C level

Table 3. Lipids profile levels in control, IHD and MI groups (Mean \pm SD).

Parameter	Control group (n=20)	IHD (n=20)	MI (n=20)
Total lipids (mg %)	628.8 \pm 42.81	850.25 \pm 50.43*	982.62 \pm 84.38*
Phospholipids (mg %)	207.8 \pm 22.84	231.25 \pm 12.84	250.8 \pm 6.44*§
Triacylglycerol (mg %)	125.9 \pm 20.67	174.3 \pm 6.891*	185.5 \pm 13.4*§
Total cholesterol (mg %)	191.95 \pm 15.22	303.6 \pm 20.35*	329.35 \pm 22.88*
HDL-C (mg %)	55.2 \pm 6.12	42.4 \pm 4.68*	41 \pm 4.21*
LDL-C (mg %)	107.35 \pm 7.35	222.5 \pm 16.2*	247.75 \pm 19.32*

Data are presented as mean \pm SD, *p <0.05 vs. control, § p <0.05 significant (IHD) vs. (MI).

and activity of protein C deficiency has a bearing with pulmonary infarction.

Van-der-Ban et al. (1996) found a reduced response to activated protein C (APC) is associated with an increased risk for cerebrovascular disease but not with an increased risk for myocardial infarction. Goto et al. (1992) reported augmented plasma protein C activity after coronary thrombolysis with urokinase in patients with acute myocardial infarction, thus, it was suggested that urokinase administration for coronary thrombolysis not only causes fibrinolysis, but also induces thrombin activity which may be antagonized by augmented intrinsic protein C activity. The diagnosis of MI is established in patients with chest pain and equivocal electrocardiogram changes by demonstrating a rise in blood levels of creatine kinase MB (CK-MB) and/or an increase in cardiac troponin I (cTnI) or cardiac troponin T (cTnT). Previous studies have shown that levels of CK-MB are increased in the left ventricle of individuals with heart disease Welsh et al, (2002).

While CK-MB as a cardiac marker depended on its relatively high concentration in heart muscle (>20%) compared to typical skeletal muscle (1–2%). There is evidence that higher concentrations of CK-MB in heart may result from ischemic stress. For example, concentrations of CK-MB have been found to be significantly higher in heart muscle of experimental animals and human myocardium with coronary artery disease, aortic stenosis, or heart failure, compared to normals. A number of studies have shown that the concentration of CK-MB is higher in ventricular myocardial tissue in animal models of hypertrophy or ischemia and in humans with a variety of cardiac conditions, compared to controls or young individuals without cardiac disease. In human myocardial biopsy material, concentrations of CK-MB have been reported to be 100-fold greater in hearts from patients with aortic stenosis, coronary artery disease, and coronary artery disease with left ventricular hypertrophy compared to patients without such findings (Welsh et al., 2002).

The study of many enzymes activities are valuable in diagnosis of many disease as the rise in the serum enzyme of CPK and CPK-MB and lactate dehydrogenase are commonly used for diagnosis of coronary heart disease. In the present study, it was found that there was a highly significant increase of serum creatine phosphor-

kinase CPK, CPK-MB and serum lactate dehydrogenase levels in IHD and MI groups as compared to the control group. The discovery of isoenzyme determination has improved the diagnostic value of enzyme tests. The cardio specific isoenzyme of CK (CK-MB) has been used successfully for the detection of myocardial infarction. Our results are in agreement with that of Welsh (2002) and Kato et al. (2006). The European Society of Cardiology (ESC) and American College of Cardiology (ACC) state that any elevation, however small, of a troponin or the creatine kinase MB (muscle, brain) isoenzyme is evidence of myocardial necrosis and that the patient should be classified as having myocardial infarction, however small (Antman et al., 2004).

Hyperlipidemia refers to increased levels of lipids (fats) in the blood, including cholesterol and triglycerides. Although hyperlipidemia does not cause you to feel bad, it can significantly increase your risk of developing coronary heart disease, also called coronary artery disease or coronary disease. People with coronary disease develop thickened or hardened arteries in the heart muscle. This can cause chest pain, a heart attack, or both (Saunders, 2007). Hyperlipidemia is a disturbance of the lipid transport system that results from abnormalities in the synthesis or degradation of plasma lipoprotein (Brown and Goldstein, 1983). There is strong evidence between abnormalities of lipids metabolism and gradual change of atherosclerosis and coronary heart disease. The substance that gives the atheroma its character is the lipid, chiefly cholesterol esters (Roheim, 1986). Measurements of plasma lipid and lipoprotein levels have been used in diagnostic medicine to assess the risk of coronary artery disease. Cholesterol and triglycerides levels have been recognized as predictors of CAD. HDL-C and LDL-C have been considered the most accurate indicators of CAD. Increased level of LDL cholesterol is associated with increased incidence of CAD.

Our study reveals a high significant increase in serum total lipids in IHD and MI compared to control group. This is in accordance with the results of Brown and Goldstein (1983). They noted the increase in serum total lipid in CAD patients. Also, it was reported that hypertension, smoking and hyperlipidemia are the most important risk factors of IHD. Saturated fat intake has been linked to an increased risk of cardiovascular disease (CVD), and this

effect is thought to be mediated primarily by increased concentrations of LDL cholesterol (Patty et al., 2010). The present study shows no significant change in phospholipids concentration in IHD group while there was highly significant increase in MI group as compared to the control group. Natio (1988) proved that the ratio of phospholipids to cholesterol ester level resulted in a corresponding change in phospholipids in similar direction.

Furthermore, triacylglycerol increased significantly in both IHD and MI as compared to the control group. It was suggested by Despres et al. (1990) that triglyceride molecules are not themselves atherogenic. High plasma triacylglycerol level may indirectly represent a cardiovascular risk factor through its effect on lipoprotein composition. Also, the results are in agreement with those of Jensen et al. (1991) who found that triglyceride level were higher in CAD patients and severity of coronary atherosclerosis has been shown to correlate better with serum concentration of triglyceride than of cholesterol.

Sigurdsson et al. (1992) suggested that elevated triglyceride levels are important as a risk factor only when associated with other lipoprotein abnormalities (elevated LDL-C or decreased HDL-C). Also Welin et al. (1991) demonstrated from follow up of incidence of coronary heart disease increased 5 fold from the lowest to the highest of value triacylglycerol. Increased serum triglycerides are a major coronary risk factor in elderly men. Moreover, Assmann (1992) suggested that triglyceridemia is a powerful additional coronary risk factor when excessive triacylglycerol coincide with a high ratio of plasma LDL-C to HDL-C.

In this study, there was a highly significant increase in serum total cholesterol in IHD and MI as compared to control group. The present study is in agreement with Kondreddy et al. (2010) who found that total cholesterol (TC) and LDL-C were significantly increased while HDL-C was significantly decreased among the CHD group. This is in accordance with the results of Bainton et al. (1992). They found that total cholesterol was higher in CAD patients than normal controls. Also, Swedarsen et al. (1991) showed that hypercholesterolemia without associated hypertriglyceridemia was the commonest abnormality. Jensen et al. (1991) observed that plasma cholesterol above the level approximately 270 mg% proportionally increased of CAD. It was concluded by Bainton (1992) that cholesterol was a more important risk factor than HDL-C and was considered to be the most important single lipid risk factor in men. The level of the plasma lipoprotein play an important role in the pathogenesis of atherosclerosis, particularly low level of HDL-C and high level of LDL-C (Roheim, 1986).

In this work the HDL-C levels showed highly significant decrease in both IHD and MI groups as compared to control group. A similar result was found by Duval et al. (1989) who reported that there was a significant decrease

in HDL in cardio vascular disease. Pometta et al. (1987) reported that HDL-C concentration correlated inversely to the development of atherosclerosis; therefore they were considered to be negative risk factor. Finally, there was a highly significant increase of LDL-C in IHD and MI groups comparing to the control group. Similar results were obtained from Henriksen (1984) who found that the lipids in atherosclerotic lesion are derived from plasma LDL. Also Jensen et al. (1991) found that LDL was higher in CAD patients than control. Avogaro et al. (1988) and Badimon et al (1992) studied LDL metabolism and proved that LDL seems to be responsible for transported approximately 70% of the total cholesterol by LDL-C the liver to the tissues.

In conclusion, this study demonstrates the association between protein C and another coagulation factor and other important risk factors as lipid profile and plasma protein C might serve as a marker for coronary artery disease in men.

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