Role of plasma homocysteine and lipoprotein (a) in coronary artery disease

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Accepted: 13 January 2004

ABSTRACT

This study looks at the possible role of some non-traditional risk factors for premature coronary artery disease (CAD) and assesses the presence of relationship between these factors and the traditional cardiovascular risk factors. The study subjects (n=45) are divided into three groups comprising 15 premature CAD patients without traditional cardiovascular risk factors (group I); 15 premature CAD patients with one or more traditional cardiovascular risk factors (group II); and 15 healthy normal control subjects matched for age and sex (group III). Estimation of plasma homocysteine (Hcy) and plasminogen activator inhibitor-1 (PAI-1) is performed by enzyme-linked immunosorbent assay (ELISA); plasma folic acid by radioimmunoassay; plasma lipoprotein a (Lpa) by turbidimetry; and plasma lipids by colorimetry. Results showed a significant association between elevated Hcy and low folate levels and premature CAD in both patient groups. Also, a significant association was seen between elevated PAI-1 and CAD in the two patient groups, and between CAD and high levels of Hcy and triglycerides, as well as a low level of high-density lipoprotein cholesterol. Lpa showed significant association with premature CAD only in group II. Thus, Hcy, folic acid and PAI-1 might serve as independent risk factors for premature CAD in patients both with and without traditional coronary risk factors. However, Lpa might confer an additional coronary risk factor only in the presence of traditional risk factors.

KEY WORDS: Coronary disease. Folic acid. Homocysteine. Lipoprotein(a). Plasminogen activator inhibitor-1.

Introduction

The term coronary artery disease (CAD) defines a disease spectrum of diverse aetiology, the common factor being an imbalance between myocardial oxygen supply and demand. Atherosclerotic plaques are the most common cause of CAD.[1] More than 200 coronary risk factors have been reported and include smoking, diabetes mellitus, hypertension, dyslipidaemia, ageing, obesity, physical inactivity and hereditary predisposition, [2] but these traditional risk factors only account for 50% of the problem.[3]

Recently, several metabolic, haemostatic and fibrinolytic factors have been shown to be involved in the pathogenesis of CAD. Among these are homocysteine, folic acid, lipoprotein (a) and plasminogen activator inhibitor-1 (PAI-1).[4]

Homocysteine (Hcy), a thiol-containing non-protein amino acid, is an intermediate in methionine metabolism. It is methylated to methionine by the transfer of a methyl group from N^5-methyltetrahydrofolate (N^5-MTHF) in a reaction catalysed by methionine synthase that requires methylcobalamin, a form of vitamin B_{12}.[5] A regulating enzyme in Hcy remethylation is methylenetetrahydrofolate reductase (MTHFR), which catalyses the reduction of N^5,N^{10}-methylenetetrahydrofolate (N^5-N^{10}MTHF) to N^5-MTHF.[6]

Hyperhomocysteinaemia may be caused by genetic or environmental factors.[7] Genetic factors are caused by deficiency of MTHFR, cystathionine ß-synthase and defects in the synthesis of cobalamin cofactors.[6] Environmental factors include folic acid, vitamin B_{12} and vitamin B_6 deficiencies, diabetes mellitus, renal failure, cigarette smoking, and the effect of drugs that interfere with Hcy metabolism.[8,9]

The possible role of Hcy in the development of CAD has not been confirmed in humans, although the induction of vascular injury by elevated Hcy levels has been demonstrated in several model systems. This may be explained by the fact that Hcy thiolactone, the reactive anhydride form of Hcy, can be converted to the sulphating coenzyme phosphoadenosine phosphosulphate. This explains the origin of the increased sulphated extracellular matrix and the growth of smooth muscle cells in developing atherosclerotic plaques.[10] The relevance of hyperhomocysteinaemia as a risk factor for CAD remains unknown.[11]

Fibrinolysis is mediated by the proteolytic enzyme plasmin, which is formed in the circulation from the inactive precursor plasminogen through the proteolytic action of the serine proteases and plasminogen activators (PAs). There are only two known physiological Pas: tissue-type PA (t-PA), and urokinase-type PA (u-PA).[12] Increased plasma level of PAI-1 results in reduced fibrinolyic activity, which predisposes to deposition of intramural and intraluminal fibrin, which potentiates micro- or macrothrombotic occlusions in

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atherosclerotic coronary arteries. However, it remains difficult to assess the possible role of PAI-1 as a risk factor for the occurrence of premature CAD.[4]

Lipoprotein-a (Lp[a]) is a cholesterol-rich plasma lipoprotein particle, the structure and composition of which closely resemble low-density lipoprotein (LDL). However, the distinguishing feature of Lp(a) is the presence of an additional large glycoprotein known as apolipoprotein (a) (apo[a]), which is linked to apolipoprotein B-100, the sole protein of LDL, by a disulphide bond. Lp (a) has a density that spans part of that of LDL and pre-ß mobility in lipoprotein electrophoresis. Apo(a) has a high structural homology with plasminogen.[13]

In the fasting state, at least 95% of apo(a) is found in lipoproteins, while the remaining 5% appears to be free and largely unassociated with lipids. Recently, increased plasma Lp(a) level has been shown to be associated with ischaemic cardiovascular disease in which both atherogenic and thrombogenic factors are implicated.[14]

The aim of the present study is to assess the possible role of plasma Hcy, folic acid, PAI-1, and Lp(a) as non-traditional risk factors among patients with premature CAD, with and without traditional risk factors such as hypertension, diabetes mellitus and cigarette smoking.

Table 1. Traditional coronary risk factors in group II CAD patients.

Patient	Risk factors
1	Hypertension, smoking, hypertriglyceridaemia, hypercholesterolaemia
2	Non-insulin dependent diabetes mellitus (NIDDM)
3	NIDDM, smoking, hypertriglyceridaemia, hypercholesterolaemia
4	NIDDM, hypertriglyceridaemia, hypercholesterolaemia
5	NIDDM
6	Smoking, hypertriglyceridaemia, hypercholesterolaemia
7	Smoking, hypertriglyceridaemia, hypercholesterolaemia
8	NIDDM
9	Smoking
10	NIDDM, hypercholesterolaemia
11	NIDDM, hypercholesterolaemia
12	NIDDM
13	Hypertension, hypercholesterolaemia
14	Smoking, hypercholesterolaemia
15	NIDDM, hypercholesterolaemia

Materials and methods

The present study included 30 patients presenting with clinical and electrocardiographic evidence of stable angina, selected from patients admitted to the cardiology unit of Alexandria Main University Hospital for diagnostic coronary arteriography. All were below the age of 45 and were divided into two groups comprising 15 patients with none of the traditional cardiovascular risk factors (hypertension, diabetes mellitus, cigarette smoking, or dyslipidaemia) and thus were said to have premature CAD (group I); 15 patients with one or more traditional cardiovascular risk factors (group II); and 15 healthy normal control subjects (as evidenced by electrocardiography) matched for age and sex (group III).

Exclusion criteria in all subjects included malignant disease, acute infection, valvular heart disease, myocarditis, cardiomyopathy, acute myocardial infarction (in the three months prior to the study), severe liver or kidney disease, and coagulopathies. Informed consent to participate in the study was obtained from all patients and controls.

All study participants provided a complete medical history and were subjected to a thorough clinical examination. Routine laboratory investigations included haemoglobin, complete blood picture, fasting plasma glucose, liver and renal function tests, serum uric acid, bleeding time, activated partial thromboplastin time (APPT), prothrombin time and thrombin time to check for acquired coagulopathies.

Specific laboratory tests included plasma homocysteine concentration by enzyme-linked immunosorbent assay (ELISA),[15] expressed as μmol/L; plasma folic acid concentration by radioimmunoassay,[16] expressed as ng/mL; plasma PAI-1 concentration by ELISA,[17] expressed as ng/mL;

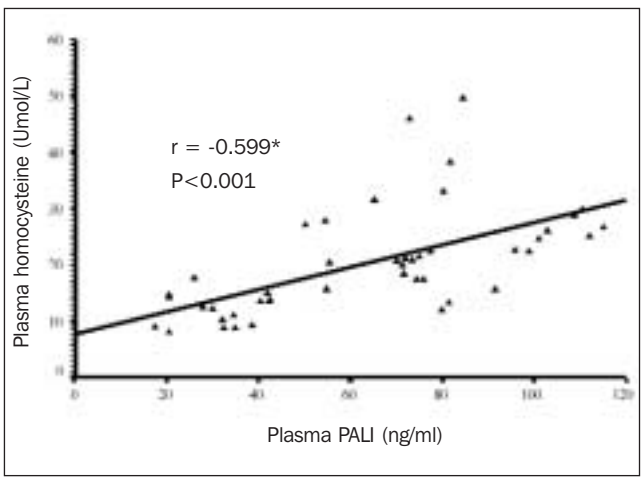

Fig. 1. Correlation between plasma PAI-1 concentration and plasma homocysteine in CAD.

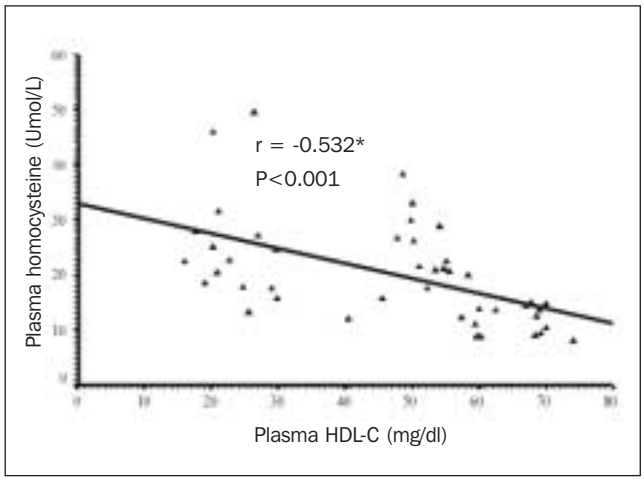

Fig. 2. Correlation between plasma HDL-C concentration and plasma homocysteine in CAD.

Table 2. Biochemical data (mean + SD [range]) in the studied groups.

Parameter	Group I (n=15)	Group II (n=15)	Control (III) (n=15)	F-test
Homocysteine (μmol/L)	23.69+6.86 [12.0-38.20]	25.41+10.36 [13.4-49.60]	11.73+2.42 [8.20-15.10]	15.6* (I,III)*,(II,III)*
Folic acid (ng/mL)	2.9 + 1.26 [0.50-5.50]	4.93 + 1.96 [0.40-6.50]	6.95 + 1.95 [4.80-10.50]	20.09* (I,II)*(I,III)*(II,III)*
PAI-1 (ng/mL)	85.67+15.88 [70.1-115.20]	73.37+23.13 [25.9-112.00]	31.81+8.76 [17.50-42.50]	41.51* (I,III)*(II,III)*
Lp (a)(mg/dL)	20.5 + 5.87 [15.30-37.60]	36.15+16.26 [20.30-85.10]	16.71+3.12 [15.00-26.10]	15.49* (I,II)*,(II,III)*
Triglycerides(mg/dL)	118.42+10.84 [105.0-145.0]	195.65+62.85 [104.0-300.0]	101.82+8.34 [87.0-117.0]	27.27* (I,II)*,(II,III)*
Cholesterol (mg/dL)	172.81+19.52 [144.6-221.7]	226.8 + 44.75 [137.0-287.0]	171.91+16.16 [134.3-195.5]	16.81* (I,II)*,(II,III)*
HDL-C (mg/dL)	51.12 + 4.46 [40.50-58.30]	23.28 + 4.46 [16.00-29.80]	65.52 + 5.14 [57.40-74.00]	313.65* (I,II)*(I,III)*(II,III)*
LDL-C (mg/dL)	98.02 + 19.39 [61.9-148.0]	166.06+40.37 [95.40-222.0]	86.03 + 15.47 [47.5-108.7]	37.33* (I,II)*,(II,III)*

*Statistically significant $P<0.001$
- Group I: CAD patients without traditional cardiovascular risk factors
- Group II: CAD patients with one or more risk factors
- Group III: control group

plasma Lp (a) concentration by turbidimetry,[18] expressed as mg/dL; plasma triglycerides (TG), cholesterol, and high-density lipoprotein cholesterol (HDL-C) concentrations by an enzymatic colorimetric method;[19–21] and serum LDL-C concentration by a polyvinyl sulphate method.[22]

Venous blood (10 mL) was withdrawn from all study participants after overnight fast (12 h). Blood was transferred to disposable tubes containing 5% ethylenediaminetetraacetic acid (EDTA). After centrifugation at 3000 rpm for 10 min, the recovered plasma was divided into two dry plastic tubes. One sample was stored at –20°C until assayed for Hcy, folic acid, TG, cholesterol, and HDL-C. The other was stored at –70°C until assayed for Lp(a) and PAI-1. The storage period was not more than four weeks.

Statistical analysis

Results were presented as mean + SD. One-way analysis of variance (ANOVA) was used to compare more than two groups, and least significant difference was used to detect significance between each two groups. Pearson's correlation coefficient (r) was used to show correlation between parameters. χ^2 test was used to compare in the studied groups. $P \leq 0.05$ was considered statistically significant.

Results

The ages in groups I and II ranged from 40 to 45 years and from 38 to 45 years, respectively (mean values 42.5 + 2.1 and 41.6 + 2.25 years, respectively). The age in the control group ranged from 38 to 45 years (mean value 41.6 + 2.25 years). There was no significant difference in the mean age values between the three groups (F=20.04, P=0.978). Men represented 73.3%, 80% and 73.3% of those in the three groups, respectively. There was no significant difference in sex distribution between the three groups (χ^2=2.696, P=0.26). Table 1 shows the traditional risk factors in group II CAD patients, while Table 2 shows statistical comparisons of the biochemical data in the studied groups. Table 3 shows correlations between the studied biochemical parameters in the patient groups.

There were significant positive correlations between plasma Hcy level and PAI-1 (Fig. 1), TG and serum LDL-C (r=0.599, P=<0.001, r=0.453, P=0.002, r=0.357, P=0.016 respectively). However, there were significant negative correlations between plasma Hcy level and that of folate and HDL-C (Fig. 2) (r=–0.707, r=–0.532, P<0.001).

Discussion

Identification of new markers associated with an increased risk of CAD may provide a better insight into the pathology of coronary atherosclerosis and facilitate the development of preventive and therapeutic measures.[23] The present study showed that plasma Hcy concentration was significantly higher in both patient groups compared to the control group. However it did not differ significantly between the two patient groups. This indicates that Hcy may be an atherogenic risk factor independent of the traditional risk factors for CAD, and agrees with the findings of previous studies.[24]

Hyperhomocysteinaemia may promote atherosclerosis and thrombosis by a number of possible mechanisms. First, oxidation of Hcy to disulphides generates superoxide anion radicals and hydrogen peroxide, resulting in inactivation of nitric oxide (NO) and endothelial cell dysfunction that may contribute to vasospasm, thrombosis, and progression of atherosclerosis.[25]

Second, hyperhomocysteinaemia may result in irreversible homocysteinylation at epidermal growth factor-like domains in fibrillin-1 and in many other extracellular proteins of the coagulation-anticoagulation and lipoprotein

Table 3. Correlation coefficient (r) between the biochemical parameters in the patient groups.

Parameter	Homocysteine	Folic acid	PLA-1	Lp(a)	Cholesterol	TG	HDL-C	LDL-C
Homocysteine								
r	–	-0.707*	0.599*	0.289	0.293	0.453*	-0.532*	0.357*
P	–	<0.001	<0.001	0.054	0.051	0.002	<0.001	0.016
Folic acid								
r	-0.707*	–	-0.631*	-0.033	-0.005	-0.148	0.184	-0.047
P	<0.001	–	<0.001	0.830	0.973	0.332	<0.001	0.761
PLA-1								
r	0.599*	-0.631*	–	0.089	0.102	0.391*	-0.508*	0.197
P	<0.001	<0.001	–	0.562	0.505	0.008	<0.001	0.193
Lp(a)								
r	0.289	-0.033	0.089	–	0.402*	0.259	-0.593*	0.528*
P	0.054	0.830	0.562	–	0.006	0.086	<0.001	<0.001
Cholesterol								
r	0.293	-0.005	0.102	0.402*	–	0.751*	-0.603	0.861*
P	0.051	0.973	0.505	0.006	–	<0.001	<0.001	<0.001
TG								
r	0.453*	-0.148	0.391*	0.259	0.751*	–	-0.735*	0.737*
P	0.002	0.332	0.008	0.086	<0.001	–	<0.001	<0.001
HDL-C								
r	-0.532*	0.184	-0.508*	-0.593*	-0.603*	-0.735*	–	-0.768*
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	–	<0.001
LDL-C								
r	0.357*	-0.047	0.197	0.528*	0.961*	0.757*	-0.768*	–
P	0.016	0.761	0.193	<0.001	<0.001	<0.001	<0.001	–

* Statistically significant at $P < 0.05$

transport pathways, with subsequent malfunctioning of such pathways.[26] Furthermore, metabolic conversion of Hcy to thiolactone may play a role in Hcy-induced vascular damage.[27]

Third, Hcy and oxidised LDL-C forms LDL-Hcy aggregates, which are precursors of the foam cell, cholesterol and lipid deposits within developing atherosclerotic plaques.[5] These aggregates enhance platelet adhesion to endothelial cells by inducing tissue factor activity (by Hcy) and up-regulating intercellular adhesion molecule-1 (ICAM-1) by oxidised LDL.[28]

Fourth, hyperhomocysteinaemia may increase factor VIIa, cause the generation of thrombin, inhibit protein C activation and may down-regulate thrombomodulin.[29] Finally, Hcy has also been seen to induce DNA damage.[30]

In the present study, plasma Hcy level in the patient groups showed a negative correlation with both plasma folate and HDL-C levels. Meanwhile, there were significant positive correlations between plasma Hcys level in the patient groups and plasma levels of PLA-1, TG and LDL-C. Such interactions between prothrombotic factors, hypofibrinolysis and dyslipidaemia may play an important pathogenic role in premature CAD.[31]

With regard to plasma folic acid, the present study showed significantly lower levels in groups I and II compared to group III (controls), and in group I compared to group II. These results were consistent with previous studies,[32,33] and indicate that folic acid deficiency could act in association with the traditional risk factors for CAD.

Folic acid has a lowering effect on Hcy because its active form, 5- MTHF, is required for the methylation of Hcy to methionine. Thus, folic acid deficiency should lead to accumulation of plasma Hcy, resulting in an increased risk of vascular disease.[33] Klerk *et al*.[34] found that impaired folate metabolism, resulting in high Hcy level, has a causal relationship to increased risk of CAD, and this was supported by the results of the present study.

It has been suggested that folic acid stimulates endogenous tetrahydrobiopterin, an essential cofactor of nitric oxide synthase (NOS) in the synthesis of NO, which mediates vasodilatation. Furthermore, it reduces superoxide anion generation by NOS and xanthine oxidase. Collectively, it is possible that hyperhomocysteinaemia may impair endothelial function by promoting oxidative stress, and that folic acid administration may prevent endothelial dysfunction through amelioration of oxidative stress.[35]

Newman[36] reported that folic acid deficiency can lead to inadequate production of S-adenosyl methionine. This may cause hypomethylation of DNA in the cells of the arterial intima, resulting in mutation and proliferation of smooth muscle cells, and possibly the formation of atheroma. Recently, Andreassi *et al*.[30] suggested that the severity of CAD is related to the MTHFR polymorphism, providing an interesting link between coronary atherosclerosis and genetic instability in humans.

The present study showed that the circulating PAI-1 levels

in both groups of CAD patients were significantly higher than in the control group. However, there was no significant difference in plasma PAI-1 levels between groups I and II. This indicates that PAI-1 may be an atherogenic risk factor independent of the presence of any of the traditional CAD risk factors, and is consistent with the results of other studies.[37]

Sobel[38] suggested that elevated PAI-1 levels may predispose to the formation of acellular thin-walled plaques that are particularly prone to rupture. High plasma PAI-1 levels may promote unstable plaque formation and reflect ongoing endothelial injury and chronic inflammation accompanying atherosclerosis.

The presence of multiple significant associations between high plasma PAI-1 level and potential atherogenic markers (e.g., Hcy) on the one hand, and some traditional cardiovascular risk factors (e.g., high plasma TG and low plasma HLD-C levels) on the other, reflect a complex interaction between these variables in the atherosclerotic process.[4,31]

The present study showed that those in group II had significantly higher plasma Lp(a) levels than those in groups I or III (controls); however, there was no significant difference between levels in groups I and III. This finding was consistent with the work of others,[39] and indicates that Lp(a) may be an atherogenic factor in CAD patients, but not in the absence of traditional risk factors.

This suggests that Lp(a) is neither an independent risk factor nor a predictor of premature CAD, and is consistent with the result of other workers who reported that increased plasma Lp(a) level is associated with ischaemic cardiovascular disease in which atherogenic and thrombotic factors are implicated.[14] However, this contradicts the result of those who reported that Lp(a) is an established independent atherogenic factor,[40] and that Lp(a) accelerates advanced atherosclerotic lesion formation and may play an important role in vascular calcification.[41]

The current study showed positive correlations between plasma Lp(a) and levels of both total cholesterol and LDL-C. High Lp(a) level increases the risk of CAD in the presence of elevated LDL-C. These findings suggest that Lp(a) level modulates the risk conferred by LDL-C and can be considered an additional risk factor.[42]

The pathophysiological role of dyslipidaemia as a traditional risk factor for CAD may be explained by several mechanisms. Hypertriglyceridaemia can reflect an increase in the concentration of lipoproteins (especially LDL). Lipoprotein lipase and phospholipase A2 (PLA2) hydrolyse lipoproteins, converting them to small, dense LDL particle. These are associated with an increased risk of CAD because they have a higher affinity for extracellular and pericellular arterial proteoglycans.[41] Furthermore, the lipid products generated by the action of PLA_2 are proatherogenic and proinflammatory precursors and they activate vascular cells to produce PAI-1, adhesion molecules, various proatherogenic cytokines and growth factors, and oxygen free radicals, leading to atherothrombotic development and plaque instability in the atherosclerotic arterial wall.[43] In addition, PLA_2 hydrolyses Lp(a) and thus increases its binding to proteoglycans and accentuates its atherogenicity.[40]

Thus, from the present study, it can be concluded that there is a significant association between high plasma Hcy, PAI-1 and the occurrence of premature CAD, which is independent of the presence of any of the known traditional coronary risk factors. An important association was found between elevated Hcy and low folate levels and CAD, suggesting that both might interact to induce endothelial dysfunction, together with impaired fibrinolysis caused by elevated PAI-1 level. In addition, dyslipidaemia may participate in the pathogenesis of coronary atherosclerosis. Furthermore, high plasma Lp(a) may play an important role in the pathogenesis of premature CAD, but only in the presence of traditional coronary risk factors.

Finally, based on the results presented here, folate supplementation is recommended for individuals who have risk factors for CAD. □

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