Lipoprotein(a) and Homocysteine Potentiate the Risk of Coronary Artery Disease in Male Subjects

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Background: Lipoprotein (Lp(a)) and homocysteine (Hcy) are independent risk factors for coronary artery disease (CAD). Hcy promotes the release of free apo(a) from Lp(a). The high fibrin affinity of free apo(a) inhibits plasminogen binding and plasmin generation. Hyperhomocysteinemia can result from a less active variant of methylene tetrahydrofolate reductase (variant C677T). Because the C677T genotype is estimated to be present in 32.2% of the Mexican population, we took advantage of this prevalence to determine the possible potentiating effect between high plasma Lp(a) and Hcy for increasing the risk of CAD in male patients.

Methods and Results: First, 222 male patients admitted for coronary angiography were recruited and classified as CAD+ or CAD-. Anthropometric measurements, traditional risk factors, and plasma total Hcy (tHcy) and Lp(a) levels were recorded in both groups. We performed a conditional logistic regression model adjusted for conventional risk factors of CAD and it became clear that Lp(a) ≥30 mg/dl was a risk factor for CAD (odds ratio [OR] 5.06, 95% confidence interval [CI] 1.88–13.51, P=0.001), whereas Hcy was not related to CAD (OR 0.44, 95% CI 0.63–2.90, P=0.44). However, when both factors were considered together in an interaction model, high tHcy and high Lp(a) plasma concentrations showed a potentiated effect (OR 10.52, 95% CI 2.18–50.71, P=0.003).

Conclusions: The combination of high Lp(a) and Hcy levels synergistically increases the likelihood of developing CAD in male patients. (Circ J 2012; 76: 1953–1957)

Key Words: Atherosclerosis; Coronary artery disease; Homocysteine; Lipoprotein(a)
cicular events in patients with CAD.13,14 Hcy is an intermediate metabolite of methionine that contributes to arterial disease through several mechanisms, such as endothelial dysfunction, increased permeability of lipid and inflammatory cells, lipoprotein oxidation, vascular inflammation, smooth muscle proliferation, platelet activation, and abnormalities in the clotting cascade.15-17 The Hcy concentration may be influenced by environmental and genetic factors, whereas changes in the plasma concentration of Lp(a) are more than 90% under genetic control.18 The frequency of the homozygous dominant mutation genotype of the enzyme methylene tetrahydrofolate reductase (MTHFR, C677T gene) is higher in the Mexican population (32.2%) and also in the Hispanic population in the United States (17.7%) compared with any other population.19 This may promote hyperhomocysteinemia and therefore susceptibility to atherosclerosis and thrombosis.

In vitro studies have shown that Hcy enhances the binding of Lp(a) to fibrin by altering fibrinolysis.20 Hcy, a reducing agent, is capable of modifying the structure and function of Lp(a) in human plasma. Nardulli et al have shown that by reducing the disulfide bonds between apo(a) and apoB-100, Hcy promotes the high affinity binding of free apo(a) to fibrin, thereby inhibiting the formation of plasmin and blocking the fibrinolytic pathway.21 Nevertheless, only a few studies have evaluated the clinical effect of the biological interaction between Lp(a) and Hcy, finding that the associated risk was greater than the 2 risks independently. However, this synergistic effect was only seen in women,22,23 and no significant interaction was seen in men.24,25 We aimed to determine the risk for CAD in men when both Lp(a) and Hcy are increased and to determine the association of Lp(a) and Hcy with other traditional coronary risk factors.

**Methods**

**Subjects**

We performed a cross-sectional study in 222 male patients admitted for coronary angiography at the National Institute of Cardiology Ignacio Chavez.

Selective coronary angiography was performed using femoral access in all patients. The severity of coronary atherosclerosis was classified as 1-, 2-, or 3-vessel disease according to the number of stenotic major coronary arteries (left anterior descending artery, circumflex artery and right coronary artery).

The patients were classified as CAD+, defined as >50% stenosis in at least 1 major coronary artery, or CAD− when they had no angiographic evidence of significant coronary artery occlusion. Patients were matched for age in pairs; if a CAD+ patient was included another CAD− patient of the same age was included too, so we had pairs of matching individuals.

We excluded patients with recent myocardial infarction or those who had been diagnosed with unstable angina less than 1 month ago, coronary bypass surgery or previous coronary intervention, thyroid disease, or kidney or liver failure because these could affect the concentrations of Lp(a) and Hcy.

For all CAD+ and CAD− patients who met the inclusion criteria, anthropometric measures and traditional risk factors were recorded. Individuals were considered to have diabetes mellitus type 2 if they had been previously diagnosed, were receiving hypoglycemic treatment and/or insulin, or had a fasting glucose level of ≥126 mg/dl on 2 or more occasions. Individuals were considered to have hypertension if they were previously diagnosed or were receiving a previously established antihypertensive therapy. Hypercholesterolemia was defined as a total cholesterol (TC) ≥200 mg/dl. Hyper-low-density lipoprotein cholesterol hypertriglyceridemia was defined as triglycerides (TG) ≥150 mg/dl and low high-density lipoprotein cholesterol (HDL-C) as <40 mg/dl or/and by a previous diagnosis or treatment.

Patients were considered to have a history of prior smoking if they had smoked 5 or more cigarettes a day and had suspended this habit for longer than 1 year. Body mass index (BMI) was calculated by a standard formula (weight (kg)/height (m)²).

The protocol was approved by the institutional ethics committee, and informed consent was given by each participant.

**Laboratory Measurements**

For each patient, we obtained a blood sample after a fast of at least 8 h. Venous blood (10 ml) was drawn and placed in a tube with EDTA as an anticoagulant. The sample was then centrifuged at 5,000 rpm for 15 min. The plasma was immediately distributed in aliquots and frozen at −70°C for less than 6 months. The samples were analyzed in blocks to reduce interassay variability.

The TC and TG were measured by enzymatic methods (Roche-Syntex/Boehringer Mannheim, Germany). HDL-C was quantified after precipitating lipoproteins containing apolipoprotein B with phosphotungstate/Mg²⁺. LDL-C was estimated using the modified Friedewald’s formula.

The plasma concentrations of total Hcy (tHcy) and Lp(a) were determined by a commercially available immunonephometer assay (Dade Behring), and the values were expressed in μmol/L and mg/dl, respectively. Hyperhomocysteinemia was assumed for tHcy ≥12.84 μmol/L (80th percentile in the CAD− group).26 [Lp(a) ≥30 mg/dl, as reported in previous studies,27-29 which corresponded to the 90th percentile in our CAD− group]).

**Statistical Analysis**

We used descriptive statistics with the mean±SD and median with interquartile range values in accordance with their distribution. Student’s t-test or Mann-Whitney test was performed to compare the differences between continuous variables according to their distribution. Significant differences between categorical variables were evaluated using the chi-square. The Pearson or Spearman correlation test was used to evaluate the association of plasma tHcy and Lp(a) with other quantitative variables. A value of P<0.05 was considered to be statistically significant.

To minimize the effects of ‘confounding’ that may have been introduced into the study sample by the different characteristics among matched pairs, a conditional logistic regression was used to quantify the association between CAD and either only high tHcy or only high Lp(a) adjusted for other traditional risk factors. In addition, an analysis of interaction was done after determining that subjects with an Lp(a) ≥30 mg/dl and tHcy ≤12.8 μmol/L had an odds ratio (OR) of 1 in the conditional model, adjusted for the traditional risk factors as well.30 The logistic regression models include the OR with a 95% confidence interval of 95% (95% CI). A value of P<0.05 was considered to be statistically significant. Statistical calculations were performed using SPSS, version 15 (Chicago, IL, USA).

**Results**

The clinical characteristics of the CAD+ and CAD− patients are shown in Table 1. Hypertension, smoking, and diabetes mellitus type 2 were much more prevalent in the CAD+ group.
than in the CAD− group. There were no statistically significant differences in BMI or blood pressure between groups.

In the CAD+ group, 52 patients had 2-vessel disease and 59 had 3-vessel disease.

The biochemical profiles of both groups are shown in Table 2. There were no differences in TC or HDL-C. However, LDL-C was higher in the CAD− group, and TG levels were higher in the patients with CAD.

CAD, Lp(a), Hcy, and Traditional Risk Factors

The plasma concentrations of Lp(a) and tHcy were higher in the CAD+ group compared with the CAD− group (Table 2). Also the Lp(a) was mildly higher in the patients with 3-vessel disease compared with the 2-vessel disease patients (30.2 ± 31.71 vs. 20.38 ± 20.88, P=0.06).

When we analyzed the effect of only high tHcy and the effect of only high Lp(a) in a conditional logistic regression model, after adjusting for traditional risk factors (Table 3), Lp(a) was shown to be an independent risk factor of CAD (OR 5.06, 95%CI 1.88–13.51, P=0.001), and tHcy was not related to CAD (OR 0.44, 95% CI 0.63–2.90, P=0.44). Hypertension, diabetes mellitus, cigarette smoking and hyper-LDL cholesterolemia were also independent risk factors for CAD, whereas the BMI and hypertriglyceridemia were not. In the interaction model adjusted for confounding factors, both factors together, Lp(a) and tHcy, increased the risk of CAD by 10-fold (OR 10.52, 95% CI 2.18–50.70, P=0.003).

Discussion

This study shows that an elevated plasma Lp(a) level is an independent risk factor for CAD in the Mexican male popula-
tion, and contrary to our expectations, tHcy had no association with CAD, although several studies have shown that Hcy is an independent risk factor for CAD,31–33 while other studies have shown conflicting results.34,35

A previous study in a Mexican population showed a high prevalence of hyperhomocysteinemia (22%) without an association with CAD36 or thrombophilia.37 Factors that may influence these results include nutrition, genetics, lifestyle, ethnicity,28 age and sex. Several studies have shown that the plasma tHcy concentration is higher in men than in women,29,40 probably due to the higher demethylation of methionine associated with higher creatinine production in males;41 sex and age could also modify the contribution of the MTHFR mutation to the tHcy concentration.32,42 Despite these findings, Balogh et al have shown that an isolated Hcy elevation had no associated risk in males.23

Depending on the microenvironment of the vascular tree, Lp(a) may change its structural characteristics and thus promote several pathophysiological mechanisms leading to the development of atherosclerosis or triggering thrombosis. In fact, it has been reported that the presence of Hcy in amounts as low as 8 μmol/L increases by 20-fold the affinity between Lp(a) and plasmin-treated fibrin.21

The polymorphism of apo(a) when incorporated into the Lp(a) lipoparticle differentially affects the fibrin-binding affinity of Lp(a). Thus, small apo(a) isoforms have a higher affinity for fibrin.44 However, when apo(a) is dissociated from Lp(a) or is produced by recombinant technology, all isoforms display a similar affinity for fibrin, independent of size, and all of them are capable of producing an important antifibrinolytic effect.45 It is important to note that the free forms of apo(a) may arise as a result of the reducing environment generated by the presence of high concentrations of Hcy.21

Both molecules influence hemostatic function either by altering the endothelium or platelet function or by favoring a thrombosis-prone condition. The presence of both Lp(a) and Hcy is a risk factor that cannot be classified as additive or multiplicative. It potentiates the risk. Lp(a) and Hcy interact to increase the risk of CAD more than 10-fold compared with a 5-fold increase when only the Lp(a) is increased.

In subjects who have elevated Lp(a) and Hcy concentrations, it is possible that a reducing environment is created that dissociates apo(a) from the Lp(a). Because this mechanism may account for the potentiating atherogenic effect of these 2 risk factors and may have implications from a clinical perspective, we expect in a projected study to demonstrate the presence of free apo(a) and its effects on fibrinolysis.

All the previous studies have seen the potentiated effect of Lp(a) and Hcy only in the female population. Balogh et al showed an OR of 3.59, whereas Foody et al described an OR of 4.82 for CAD, contrary to the previous finding of higher homocysteine in men than in women.39

Our study found this interaction also in the male population, probably due to the special characteristics of our ethnic group, which has the highest prevalence of the MTHFR mutation than any other population worldwide,19 and the dietary characteristics of the Mexican population in regard to the low consumption of vitamin B12 and folic acid, although it was not possible to assess these variables in our present work. Despite the genetic and ethnic character of the high Hcy reported here, we think that the Lp(a)/Hcy effects on CAD here reported can be extended to other populations that may have combined increased Lp(a) and Hcy.

Based on these conclusions, study of the interaction between Hcy and Lp(a) consequently becomes a priority for research into diseases with a significant impact on public health. We believe that these findings highlight the importance of identifying individuals with the dual risk factor of elevated Hcy and Lp(a) to focus on preventive measures that might decrease the risk of atherothrombotic disease. Hcy levels are known to be decreased by the consumption of folate, vitamin B6 and B12, and Lp(a) can be modified by the use of nicotinic acid.

Study Limitations

We have several limitations in our study, including the design of a cross-sectional study, that the high-risk population studied was referred to a high specialty center, and also the lack of evidence, with a functional approach of the fibrinolytic impairment caused by tHcy and Lp(a) interaction as Nardulli et al have done in vitro.

Even though the small number of patients studied could be seen as a limitation, we performed sample size calculations for the cases and controls. Lp(a) was calculated based on the frequency of 30% for patients with coronary atherosclerosis based on a previous study in the same population,35 with an alpha error of 0.05 and a beta error of 0.20, yielding a sample size of 81 individuals per group.

A strength of our study is that contrary to previous studies, our population was selected to include only stable CAD patients to reduce the bias of an increased Lp(a) and Hcy concentration caused by an acute myocardial infarction, and all the patients had CAD confirmed by coronary angiography.

Conclusions

Lp(a) is an independent risk factor for CAD in the studied population. There is an interaction between Lp(a) and Hcy that significantly potentiates the risk of CAD.

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Disclosure

Conflicts of Interest: The authors state that they have no conflicts of interest.

References


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32. Supplementary Files

33. Supplementary File 1

34. Figure S1. Distribution of Lp(a) and homocysteine level in the CAD+ group.

35. Figure S2. Distribution of Lp(a) and homocysteine level in the CAD− group.

Please find supplementary file(s); http://dx.doi.org/10.1253/circj.CJ-12-0039

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