Gender Differences in Thrombogenic Profile Associated to Coronary Obstruction Angiographically Evaluated

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Abstract: *Objective*: To identify potential differences between concentrations of thrombogenic markers associated to coronary obstruction in men and women.

Patients and Methods: We included 502 consecutive patients (251 men and 251 women), with a mean age of 63.94(11.26) and 66.08(10.58) years respectively. In all patients coronary arteriography was performed, either by presenting an acute coronary episode or for discard coronary heart disease (CHD). CHD was defined as a ≥50% narrowing of lumen in one of the three major coronary arteries. At the hospital admission, a blood sample was withdrawn to determine lipoprotein (a), fibrinogen, C reactive protein (CRP) and D-dimer levels. Patients also answered a survey questionnaire which included family and personal history of cardiovascular disease, and personal habits. Weight, height, and abdominal perimeter were also measured.

Results: 80.47% of men and 59.36% of women presented coronary heart disease. Hypertension, diabetes and dyslipidaemia were clearly associated with CHD in women whereas in men only dyslipidaemia and diabetes showed this association. D-dimer was the only marker with significantly higher values ($p<0.032$) in men with coronary obstruction, while in women these markers were CRP ($p<0.004$), Lp(a) ($p<0.001$) and fibrinogen ($p<0.045$). In the multivariate analysis, hypertension, dyslipidaemia, smoking habit and Lp(a) showed an independently association with CHD in women, whereas in men dyslipidaemia and age were the independent risk factors for CHD.

Conclusions: Our results strongly suggest that major cardiovascular risk factors associated to CHD present gender differences. Lipoprotein (a) proved to be the only differential thrombogenic factor between men and women independently associated with coronary obstruction.

Keywords: Coronary heart disease, thrombogenic markers, gender differences.

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in Europe, with 1.9 million deaths each year, greater in women (55%) than men (43%) [1]. Almost half of all deaths are attributed to coronary heart disease (CHD) and one third to stroke [2].

Coronary heart disease (CHD) can occur in a different way in males and females, pathophysiological mechanisms are different and the risk-benefit balance in more commonly accepted treatments may not be the same [3-5]. Onset of CHD in women occurs at approximately a decade later than men, with a greater risk profile and worse prognosis. Despite the importance of this problem for women's health, the specific characteristics of clinical manifestations, treatment and prognosis are poorly studied, largely due to an insufficient presence of women in clinical trials.

Although there are very few studies specifically focused to female population, the classic and most important risk factors (dyslipidemia, smoking, diabetes and high blood pressure) are similar in both sexes, but differences are established mainly in diabetes mellitus and dyslipidaemia. Diabetes mellitus is a more powerful predictor of cardiovascular risk in women than in men. Low values of cholesterol associated to high density lipoprotein (HDLc) and high triglyceride concentrations have especially negative connotations in women. Simultaneous use of contraceptives and tobacco increases risk with a powerful thrombotic effect [6,7]. In the last decade, advances in understanding of CHD physiopathology have led to identify several thrombogenic [lipoprotein (a), fibrinogen, D-dimer] and inflammatory (C reactive protein, CRP) risk factors with a different role in CHD development, either activating coagulation, interfering in fibrinolysis or reflecting dissemination of atherosclerotic process.

The objective of our study was to determine possible gender differences in several of this thrombogenic and inflammatory markers associated with coronary obstruction.

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PATIENTS AND METHODS

We studied 502 patients (251 males and 251 women) sent to the Basurto Hospital Haemodynamic Laboratory, for cardiac catheterisation following an acute coronary episode or to diagnose coronary artery disease. None had taken antithrombic medication in 6 hours prior to the study. In all patients, coronary arteriography was performed *via* the femoral artery using the Judgkins technique, catheterising the left ventricle and the three main coronary trunks. Presence of coronary artery disease (CHD+) was established when obstruction in one of the major vessels was greater than 50%. Normal group (CHD⁻) group was made up of symptomatic patients sent to the Coronary Unit to diagnose obstructive coronary disease but whose coronary angiograms turned out to be normal.

A survey questionnaire about smoking and drinking habits, as well a clinical history and treatments for high blood pressure, dyslipidaemia and diabetes was performed. Weight and height were taken to calculate Body Mass Index (BMI). Patients with dyslipidaemia were defined as patients with abnormal lipid levels in the first or previous analysis and who were under lipid lowering drugs therapy. Hypertensives were patients with high blood pressure levels on admission to hospital, or who were under hypertensive treatment regardless of current blood pressure levels. Diabetes group was defined as patients with basal glucose levels >1.26 mg/dl or under diabetic treatment regardless of current glucose levels. The cohorts of males and females examined in this study were ethnically homogenous

On admittance, previous to coronariography, blood samples were withdrawn to determine the following parameters: Lipoprotein (a) [Lp(a)], C-reactive protein (CRP), fibrinogen and D-dimer levels. Several aliquots of the remainder of each specimen were then frozen at –70º C and stored for future analyses. CRP was determined by high sensitivity assay (Tina-Quant), an immunoturbimetric latex particles test (mouse anti-CRP monoclonal antibody-coated latex particles), with measurement intervals of 0.10-20 mg/l, and an interseries coefficient of variation (CV) of 5.70% (x=0.55 mg/L) and 2.51% (x=12.36 mg/L). D-dimer (Biomerieux) sandwich immunoenzyme assay was used to determine D-dimer levels, following the ELFA (Enzyme Linked Fluorescent Assay) technique, with a measurement interval of 45-10000 ng/ml, and an interseries CV of 5.7% (x=264 ng/ml) and 7.1% (x=7283 ng/ml). Using immunonephelometry (Instrumental Laboratories I.L.), we measured fibrinogen according to Clauss's method. Lp(a) was determined by ELISA (TintElize Lp(a), Biopool), with a measurement interval of 0-60 mg/dl and an interseries CV of 7.7% (x=10 mg/dl) and 2.7% (x=40 mg/dl).

The study protocol was accepted by the Clinical Trials Committee of the Basurto Hospital and explained to the patients, who freely agreed to be included in the study, signing the informed consent approved by the committee.

Data Analysis

Parametric laboratory values were expressed as means and standard deviations and non parametric as medians and interquartile (Q1-Q3) ranges. To evaluate the differences in the means of quantitative variables we used analysis of variance (ANOVA) and Bonferroni for parametric variables, and for non-parametric variables the Kruskall-Wallis test and Mann-Whitney's U test. Categorical variables were compared using the χ^2 test. In all cases, a value of p< 0.05 was considered significant. Statistical analysis was performed using the SPSS v 17.0 software package.

RESULTS

251 women and 250 men with an average age of 67.27(11.05) and 64.35(11.29) years respectively, were studied. 150 women (59.6%) presented coronary artery disease (CHD+), while this prevalence in males was 80.9% (203 patients), difference statistically significant (p< 0.001).

Comparing baseline characteristics between men and women, we found statistically significant differences in age (p< 0.005), alcohol, tobacco consumption (p<0.001) and prevalence of hypertension (p< 0.001), but not with BMI, although average values obtained in both sexes were overweight (Table **1**).

Table 1.   **Clinical and Demographic Characteristics of the Population Studied**

	Men (N=251)	**Women (N=251)**	**p**
Age	63.94(11.26)	66.68(10.58)	0.005
BMI(Kg/m^2)	27.63(3.50)	27.97(4.61)	0.359
Alcohol (g/week)	201.77(219)	101.38(82.81)	0.001
Smokers	27.3%	12.9%	0.001
Dyslipidaemia	62.8%	59.8%	0.495
Hypertension	50%	72%	0.001
Diabetes	25.9%	32.6%	0.107

BMI: Body mass index.

Table **2** shows concentrations of inflammatory and thrombogenic markers studied in both sexes. Women showed higher and statistically significant values of Lp(a) (p<0.004) and fibrinogen (p< 0.001).

Table 2.   **Statistical Analysis of Differences in Thrombogenic and Inflammatory Markers. Total Sample**

	Women (N=251)	**Men (N=251)**	**P**
D-dimer (ng/ml)	447(306-708)	433(273.75-900)	ns
CRP (mg/L)	3.29(1.28-8.45)	4.21(1.52-12.53)	ns
Lp(a) (mg/dl)	11.69(3.07-28.17)	7.93(1.46-21.59)	0.004
Fibrinogen (mg/dl)	41.36 (117.0)	358.91(93.31)	0.001

Parametric variables are expressed as means (SD); p values for differences between groups were determined by t Student test; non parametric variables are expressed as medians and interquartile ranges; p values were determined using Mann-Whitney U test.

Patients were distributed in two groups, depending on the presence or absence of coronary artery disease (CHD+ and CHD-, respectively); its clinical and demographic characteristics are shown in Table **3**. Women and men CHD+ showed high and significant prevalences of dyslipidaemia and diabetes mellitus, while in women only

Table 3.   Clinical and Demographic Characteristics for Women and Men with and without Coronary Obstruction

	Women			Men		
	CAD+	CAD-	p	CAD+	CAD-	p
Age	67.27 (11.05)	65.86 (9.89)	ns	64.35 (11.29)	62.21 (11.08)	ns
BMI (kg/m^2)	27.96 (4.59)	27.99 (4.65)	ns	27.46 (3.52)	28.34 (3.34)	ns
Alcohol (g/week)	109.87 (87.85)	87.22 (73.85)	ns	203.72 (213.9)	193.58 (242.75)	ns
Smokers	15.5%	9%	ns	28.8%	21.3%	ns
Dyslipidaemia	69.2%	46%	0.001	65.5	51.1%	0.065
Hypertension	79.9%	60.4%	0.001	51.5	43.8%	ns
Diabetes	38.2%	24.5%	0.026	28.6%	14%	0.047

CAD+: with coronary artery disease; CAD-: without coronary artery disease.
BMI: body mass index.
Quantitative variables are expressed as means (SD); p values for differences between groups were determined by t Student test. Qualitative variables are expressed as %; p values for differences between groups were determined by X^2 test.

arterial hypertension differed significantly between the two groups (p< 0.001).

In relation to inflammatory and thrombogenic biomarkers, women with CHD+ showed higher and statistically significant values of CRP (p<0.004), Lp (a) (p <0.001) and fibrinogen (p< 0.045), while in males D-dimer was the only parameter associated to CHD (p< 0.032) (Table **4**).

Multivariate analysis (Table **5**) adjusted for diabetes, CRP, D-dimer and fibrinogen points out that in women high blood pressure, dyslipidaemia, tobacco and Lp(a) are independently associated with CHD whereas in men the independent factors are age and dyslipidaemia.

DISCUSSION

In Spain, coronary artery disease is the first cause of mortality in men and the second in women. Increase of cardiovascular mortality with age in women is quite superior to total mortality, notably in case of ischemic heart disease, from 8/100.000 (45-54 years) to156/100.000 (65-74 years) [8].

As expected, in our study the prevalence of CHD was higher in men, while the average age was higher in women confirming that women develop CHD with temporal delay respect to men.

Regarding major cardiovascular risk factors, some differences between sexes have been found: women presented higher prevalence of arterial hypertension-arterial blood pressure increases with age more powerfully than in men, so from 60 years the prevalence of hypertension is greater in women. A lower consumption of tobacco and alcohol and an increased prevalence of diabetes were also found in women. Many studies [9-11] have shown that at the onset of coronary heart disease, women have worse prognosis than men, partly due to a major accumulation of risk factors: an older age and a higher presence of hypertension and diabetes.

D-dimer is a primary degradation product of crosslinked fibrin, and elevated D-dimer levels suggest ongoing thrombin generation and breakdown. Vijay G Kalaria *et al*. [12] studied 791 men and 254 women with myocardial infarction and found that high levels of D-dimer were associated with an increased risk of recurrent coronary

Table 4.   Statistical Analysis of Differences in Thrombogenic and Inflammatory Markers. Women and Men with and without Coronary Obstruction

	Women			Men		
	CAD+	CAD-	p	CAD+	CAD-	p
D-Dimer (ng/ml)	472.5 (334.5-808.5)	426 (267-657)	ns	453 (285.75- 921.75)	349.5 (233-263)	0.032
CRP (mg/L)	4.28 (1.57-11.24)	2.52 (1.17-5.6)	0.004	4.16 (1.52-12.53)	4.26 (1.53-12.95)	ns
Lp(a) (mg/dl)	20.1 (6.59-35.16)	5.3 (1.48-16.34)	0.001	8.05 (1.76-23.31)	4.05 (1.11-19.84)	ns
Fibrinogen(mg/dl)	425.86 (123.85)	394.86 (103.95)	0.045	358.96 (95.02)	358.72 (86.66)	ns

events in men but not in women. In our study high levels of D-dimer were significantly associated with coronary obstruction only in men.

CRP, fibrinogen and Lp(a) values were significantly higher in women with CHD, while in men only Lp(a) values were high, although without statistical significance. Several authors [13,14] have demonstrated that CRP predicts cardiovascular risk in women, inflammation may act not only promoting atherogenesis but also destabilizes the vulnerable atherosclerotic plaques. Regarding fibrinogen, all studies agree that menopause markedly increases fibrinogen concentrations, which would be one of the factors that contribute to enhance cardiovascular pathology in postmenopausal women [15-17].

Table 5. **Biomarkers Independently Associated to Coronary Arterial Disease (CAD+) in Men and Women. Multivariate Analysis[*]**

		OR	CI	p
Women	Lipoprotein (a)	1.059	1.034-1.084	0.000
	Dyslipidemia	2.516	1.355-4.673	0.003
	Hypertensión	3.001	1.473-6.114	0.002
	Smokers	2.663	1.021-6.946	0.045
Men	Dyslipidemia	2.612	1.245-5.480	0.011
	Age	1.041	1.005-1.079	0.025

OR: odds ratio; CI: confidence interval of 95%.
*Model adjusted for diabetes, fibrinogen, d-dimer and CRP.

Lp(a) lipoprotein is a low-density lipoprotein particle in which apolipoproteín B-100 is linked by a single interchain disulfide bridge to a unique glycoprotein, apoprotein(a) [18,19]. Although the mechanism is not clearly established, elevated Lp (a) values had been considered an independent risk factor for the atherosclerosis and thrombosis pathophysiological process in CHD [20]. Jacqueline Suk Danik *et al.* [21] in a prospective study that assessed 27.791 healthy women in a follow-up of 10 years, found that women with Lp (a) levels >30 mg/dl were more sensible to suffer coronary problems or stroke; if levels were ≥65 mg/dl, the probability to develop a heart attack or an ischemic stroke or to need cardiovascular intervention (surgery or angioplasty) or even to die from cardiovascular disease, increases a 67%, compared with women who had low Lp (a) levels. Women with Lp (a) concentrations exceeding 65 mg/dl and LDLc levels≥120 mg/dl enhanced the risk to develop the same clinical events to 80%.

Results of the multivariate analysis have confirmed that Lp (a), arterial hypertension, dyslipidaemia and cigarette smoking were the most powerful factors to predict CHD in women's group due to they were independently related to CHD. Diabetes, D-dimer and fibrinogen are associated with an increased risk of coronary heart disease in women, but this relationship lost significance in the multivariate

adjustment, when other risk factors are taken into account. In males, dyslipidaemia and age were independently associated with CHD.

Our results strongly suggest that major cardiovascular risk factors associated to CHD present gender differences. Lipoprotein (a) proved to be the only differential thrombogenic factor between men and women independently associated with coronary obstruction.

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