

Gender-related differences in oxidative stress levels among elderly patients with coronary artery disease

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Objective: To evaluate whether gender-related differences exist concerning oxidative stress levels in aged patients with coronary artery disease (CAD).

Design: Case-control.

Setting: Clinical and research center.

Patient(s): Elderly subjects of both genders with or without CAD.

Intervention(s): None.

Main Outcome Measure(s): Serum hydroperoxides (HP) as index of oxidative stress levels.

Result(s): The HP levels were comparable in aged control subjects of both genders (376 ± 20 arbitrary units [AU] in women, 333 ± 19 AU in men) but significantly increased in CAD (456 ± 15 AU) compared with all control subjects (357 ± 14 AU). Moreover, among CAD patients, the HP levels were higher in women than in men (536 ± 33 AU and 428 ± 15 AU, respectively). Multivariate analysis, in which CAD represented the dependent variable, indicated that dyslipidemia was independently associated with CAD in men (odds ratio [OR] 5.8), whereas HP >50th percentile represented the only strong independent risk factor for CAD in elderly women (OR 8.4).

Conclusion(s): Differences in oxidative stress levels between elderly males and females might provide a biochemical basis for the epidemiologic differences in CAD, which might help to open new opportunities in the management of patients with cardiovascular disease from a gender point of view. (Fertil Steril® 2007; ■: ■ - ■. ©2007 by American Society for Reproductive Medicine.)

Key Words: Gender differences, menopause, CAD, oxidative stress

The aging process is associated with increased prevalence of cardiovascular and neurodegenerative diseases and cancer (1). Although traditionally viewed as separate disease entities, accumulating evidence indicates that there are similar physiopathologic mechanisms underlying these conditions. In particular, it is recognized that oxidative stress represents a pivotal role in all of these disorders. Moreover, it is otherwise known that gender-related differences exist concerning incidence of illness and oxidative stress levels in subjects who develop neurodegenerative disease or in some types of cancer (2–4). Specifically, elderly women are at higher risk of developing Parkinson and Alzheimer diseases and showed higher levels of oxidative stress than male patients (2, 3). On the other hand, epidemiologic evidence from a number of studies suggests that women are more susceptible to tobacco-induced carcinogenesis than men, and recent results indicate that a higher level of lung cytochrome P4501A1 (CYP1A1) gene expression in women plays a significant

role in lung DNA adduct formation and supports a higher susceptibility to lung cancer among women (5, 6).

Interestingly, a relationship between cardiovascular disease and lung cancer in women, through oxidative stress and free radical generation associated with the atherosclerotic process, has been postulated (4). Specifically, epidemiologic data from a study conducted in a large cohort of patients with previous atherosclerotic disease indicates an association between atherosclerosis and lung cancer in women (4). A fivefold increased risk ratio of cancer in atherosclerotic compared to nonatherosclerotic women was reported, which remained significant after adjustment for smoking habit and other potential confounders. The authors did not observe this relationship for male subjects and hypothesized that oxidative stress due to atherosclerosis may increase the risk of cancer in atherosclerotic women because of a gender-specific susceptibility to oxidation.

Unfortunately, studies evaluating the risk of cardiovascular disease in elderly women which include markers of oxidative stress are lacking (7). In particular, it is not clear whether gender-related differences exist concerning oxidative stress levels in aged patients with coronary artery disease (CAD).

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The estimation of serum hydroperoxide (HP) levels may represent an index of oxidative stress, because hydroperoxides are primary oxidation products, generated by oxidative damage against a variety of biologic substrates, including proteins, lipids, nucleic acids, or carbohydrates. Such biochemical alterations may provoke marked changes in the cellular phenotype and contribute to the onset and progression of atherosclerotic lesion and cardiovascular disease. Therefore, in the present study, we aimed to evaluate whether differences exist in the levels of HP in a cohort of elderly subjects of both genders with or without CAD.

MATERIALS AND METHODS

Recruitment of patients and controls was conducted as previously described (8, 9). In the present study, 51 men and 18 women with angiographically verified CAD were enrolled. Moreover, 13 men and 17 women were included as control subjects. Control subjects underwent an accurate medical check-up, which included a detailed history collection, and health screening test, including a physical examination, electrocardiogram, and laboratory analysis. All control subjects had no history of definite or suspected CAD and were evaluated for the presence of risk factors for atherosclerosis.

All enrolled subjects gave a complete history concerning their previous medical history, the presence of cardiovascular risk factors, and current medications, which included nitrates, oral aspirins, calcium antagonists, angiotensin-converting enzyme inhibitors, and diuretics. Smoking habit was coded into current smokers, ex-smokers (since at least 6 months), and never smokers. Subjects with blood pressure >140/90 mm Hg or using antihypertensive were defined as hypertensive; those presenting a history of diabetes, receiving antidiabetic therapy, or with confirmed fasting glycemia >126 mg/dL, were considered to be diabetic; and individuals with total cholesterol concentration \geq 220 mg/dL, with triglyceride concentration \geq 200 mg/dL, or under lipid-lower-

ing therapy were classified as dyslipidemic. All subjects were free from acute or chronic inflammatory disease, immunologic disease, and history or evidence of malignancy, and none were receiving hormone replacement treatment or vitamin and/or antioxidant therapies.

Fasting blood samples were obtained, kept on ice, and centrifuged (2500g, 10 minutes, 4°C) within 15 minutes after blood collection, and serum aliquots were prepared and stored at -80°C until assayed with the automatized version of the D-Roms test (Diacron, Grosseto, Italy), whose performance and analytical characteristics we have previously evaluated (10). The results were expressed as arbitrary units (AU).

Fully informed consent was obtained from each subject entering the study, and the experimental protocol was approved by the local Hospital Ethics Committee.

Data were expressed as mean \pm SEM, unless otherwise stated. Statistical analysis performed included Student *t* test, χ^2 test, analysis of variance, and Scheffe test. The logistic regression analysis was performed to determine independent correlates of CAD in both sexes. Hydroperoxide levels were categorized according to the 50th percentile (corresponding to 402 AU for women and 366 AU for men) for univariate and multivariate analysis. A *P* value of \leq .05 was considered to be statistically significant. Analyses were performed using the statistical package Statview, version 5.0.1 (Abacus Concepts, Berkeley, CA).

RESULTS

Clinical and demographic parameters in the study subjects are summarized in Table 1. There were no significant differences among groups regarding prevalence of diabetes, hypertension, and dyslipidemia. However, prevalence of subjects with smoking habit was significantly higher in men (*P* < .01).

TABLE 1

Demographic and clinical characteristics of subjects.

	Men		Women	
	Control	CAD	Control	CAD
n	13	51	17	18
Age (yrs) (mean \pm SD)	68 \pm 8	69 \pm 9	67 \pm 7	70 \pm 9
Hypertension	7 (54)	29 (57)	10 (59)	11 (61)
Diabetes	1 (8)	17 (35)	3 (18)	6 (33)
Dyslipidemia	4 (30)	34 (66)	6 (35)	13 (72)
Never smokers	11 (85)	27 (53)	15 (88)	16 (89)
Ex-smokers	2 (15)	19 (37)	2 (12)	2 (11)
Current smokers	0 (0)	5 (10)	0 (0)	0 (0)

Note: Data are n (%) unless stated otherwise.

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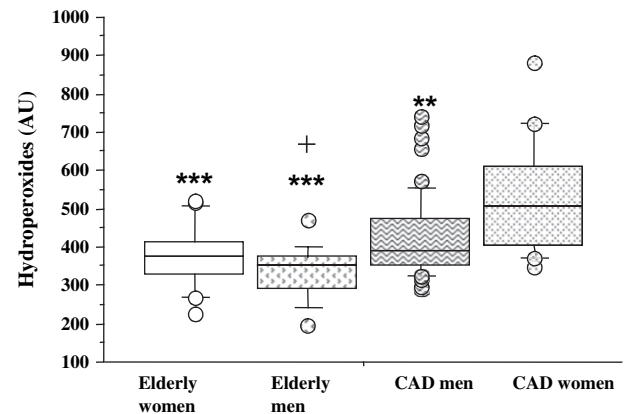
Serum concentration of HP was 376 ± 20 AU in control women, 333 ± 19 AU in control men, 428 ± 15 AU in men with CAD, and 536 ± 33 AU in women with CAD ($P < .001$). Data obtained indicate that no significant difference resulted regarding HP levels between control women and men (Fig. 1). However, HP was significantly increased in patients with CAD compared with control subjects (Fig. 1). Moreover, among patients with CAD, HP was significantly higher in women than in men (Fig. 1).

Levels of HP depending on the presence of CAD and other determinants for atherosclerosis in women and men are presented in Table 2. Results indicated that for women HP was significantly higher in subjects with diabetes, dyslipidemia, or CAD. Moreover, in men HP was increased in subjects with diabetes, smoking habit, or CAD.

On univariate analysis, in which CAD represented the dependent variable, presence of dyslipidemia and HP >50th percentile were risk markers of CAD in women, whereas smoking habit and extent of dyslipidemia predicted CAD in men (Table 3). The multivariate analysis indicates that dyslipidemia was independently associated with CAD in men, whereas HP >50th percentile represented the only strong independent risk marker of CAD in elderly women (Table 4).

FIGURE 1

Levels of hydroperoxides in elderly women and men with and without coronary artery disease (CAD). Median, interquartile, outliers, and extremes of hydroperoxides are given. AU = arbitrary units. ** $P < .01$, *** $P < .001$ vs. CAD women; + $P < .05$ vs. CAD men.



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TABLE 2

Levels of hydroperoxides depending on presence of coronary artery disease (CAD) and other determinants for atherosclerosis.

Predictor	Women		Men	
	Hydroperoxides (AU)	P	Hydroperoxides (AU)	P
Age		.88		.87
<69 yrs	462 ± 42		407 ± 16	
≥ 69 yrs (50th percentile)	455 ± 22		411 ± 21	
Type 2 diabetes		.04		.029
Absence	431 ± 27		390 ± 16	
Presence	538 ± 45		454 ± 21	
Hypertension		.42		.72
Absence	482 ± 39		415 ± 24	
Presence	442 ± 30		405 ± 15	
Dyslipidemia		.003		.12
Absence	384 ± 20		384 ± 22	
Presence	521 ± 35		427 ± 17	
Smoking		.9		.006
Current and Ex-smokers	457 ± 26		453 ± 22	
Never smokers	466 ± 53		379 ± 15	
CAD disease		.0003		.004
Absence	376 ± 20		333 ± 19	
Presence	536 ± 33		428 ± 15	

Note: Data are expressed as mean ± SE. AU = arbitrary units.

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TABLE 3

Risk factors for CAD in univariate analysis.						
	Women			Men		
	OR ^a	95% CI	P	OR ^a	95% CI	P
Age	1.05	1–1.13	.28	1.02	1–1.1	.6
Diabetes	2.3	0.9–11	.3	6.5	0.8–54	.08
Dyslipidemia	4.8	1.1–20	.03	6.7	1.6–27	.009
Hypertension	1.1	0.7–4.3	.9	1.2	0.7–42	.7
Smoking habit	1	0.5–7.5	.9	4.9	1–24.3	.052
Hydroperoxides >50th percentile	12	2.3–55	.002	2.7	0.9–10	.13

Note: CAD = coronary artery disease; CI = confidence interval; OR = odds ratio.
^a Control subjects as reference.

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DISCUSSION

In the present study, we aimed to determine whether any gender-related difference exists concerning oxidative stress in elderly patients with CAD.

It is well known that young women during their fertile life are at lower risk of cardiovascular events compared with men, being protected by estrogen action (11). It is also known that oxidative stress is generally higher in men than in premenopausal women (12). However, after menopause the risk of experiencing cardiovascular events rapidly rises in women (11). In parallel, levels of biomarkers of oxidative stress rapidly increase in postmenopausal women, and this increase might be related to greater risk for cardiovascular disease in elderly women (13, 14). In the present population, we enrolled elderly women, almost all in a postmenopausal status for many years, and this condition might significantly affect oxidative stress levels, as suggested by other observations of increased levels of oxidant damage in women after menopause (15, 16). In line with that evidence, we did not find any significant difference concerning HP levels between women and men in our control group which also included elderly

subjects (mean age 69 years). Moreover, although increased in all CAD patients compared with controls, we found that oxidative stress appears even more important in female patients and represents the only significant predictors of CAD in the women, suggesting a different vulnerability toward oxidative stress between male and female CAD patients. This was despite the fact that the group of CAD women enrolled in the present study presented less severe disease than CAD men (55% and 45% of the women with monovessel and multivessel disease and 25% and 75%, respectively, of the men), and none were current smokers.

Oxidative stress has a major role in the pathogenesis of different chronic and degenerative conditions, including aging and atherosclerosis, and it has been correlated to practically all of the risk determinants relative to coronary artery disease, such as diabetes and hypercholesterolemia (8, 17). However, to the best of our knowledge, the present study is the first to report a gender-related difference concerning oxidative stress in elderly CAD patients. This finding may have importance, because although cardiovascular disease (CVD) represents the leading cause of death and disability in both women

TABLE 4

Multivariate model predicting CAD.						
	Women			Men		
	OR ^a	95% CI	P	OR ^a	95% CI	P
Dyslipidemia	2	0.8–11	.4	5.8	1.4–25	.017
Smoking habit				4	0.9–21	.1
Hydroperoxides >50th percentile	8.4	1.5–45	.014			

Note: Abbreviations as in Table 3.
^a Control subjects as reference. Odds ratio derived from logistic regression analysis including the variables listed in the table.

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and men over age 50, the influence of gender on CVD has been misperceived and understudied (18). In fact, the lower incidence of cardiovascular events observed in premenopausal women dramatically raises after menopause to reach and eventually overcome that of men in advanced age (19). Yet aspects related to pathogenesis, clinical presentation, and outcome of CVD have not been extensively studied from a gender point of view. Among them, the role of oxidative stress is particularly interesting. It represents a shared pathologic determinant not only for cardiovascular disease and atherosclerosis but also for different chronic and progressive conditions, such as cancers and neurodegenerative processes. Some suggestions may arise from data obtained in these other critical illnesses, for which results are already available testifying that elderly women are more prone to the effect of oxidant species than men, evidence that may be associated with the increased risk for certain diseases in women (2, 3). In particular, in subjects with neurodegenerative disease, it is known that elderly female patients present higher levels of oxidative DNA damage and biomarkers of lipid peroxidation than men (2, 3). Although still debated, one issue in lung carcinogenesis is that women may be at higher risk of smoking-associated lung cancer than men, and variations in carcinogen cellular fate may contribute to gender-related differences in the association between smoking and risk (5, 6),

In the field of cardiovascular disease and related conditions, an interesting study has been conducted in type 1 diabetes patients with a short duration of disease and good metabolic control (20). Results obtained clearly indicate that women showed reduced antioxidant capacity together with significantly increased levels of lipid HP compared with not only nondiabetic subjects of both genders but also male patients, suggesting a higher and severe alteration of the oxidative pattern in diabetic females (20). This evidence also finds a correspondence in the present study, because, although in our population the prevalence of diabetes or dyslipidemia between sexes was not different, in those subjects with diabetes or dyslipidemia, women showed elevated levels of hydroperoxides compared with men ($P < .05$ and $P < .01$, respectively).

The relatively small sample size may be considered a potential limit of the present study. However, although the results need to be confirmed in larger studies, this is the first one to show gender-linked differences in the oxidative stress status among elderly CAD patients. Dissimilar levels for various other cardiovascular biomarkers in relation to gender have been already observed among men and women with the same degree of disease (21, 22). At the moment, the reasons for the overwhelming gender disparity concerning oxidative stress in these conditions are unclear but are presumably linked to the hormonal status and associated with a reduction of DNA repair mechanisms, as some authors have suggested (4, 6). Thus, on the basis of these observations, it is conceivable to identify critical areas of further inquiry concerning the oxidative process in elderly women

related to CVD. In fact, additional knowledge in this field might help in the advancing of this new gender-related understanding into improved outcomes for women and consequently lead to the discovery and adaptation of radically different approaches to diagnosis and management of CVD in women when appropriate.

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