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Short article

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The Effect of Low Testosterone and Estrogen Levels on Progressive Coronary Artery Disease in Men

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Abstract

Background: Age-related morbidity and mortality rates from coronary heart disease (CHD) are higher in men than in women. Abnormal androgen levels cause a variety of abnormal symptoms in men. Testosterone and estrogen are the main sex hormone in men and women, respectively, and studies have shown that they have important roles in cardiovascular health and disease.

Methods: We measured testosterone and estrogen in 102 men with coronary heart disease and 45 controls. Blood samples were collected from subjects and plasma testosterone and estrogen were measured by ELISA.

Results: Men with coronary heart disease had less testosterone (OD Ratio: 0.782) and estrogen (OD Ratio: 0.955) than controls.

Conclusions: Low testosterone and estrogen levels correlate with coronary artery disease.

Keywords: Coronary artery disease, Estrogen, Testosterone.

Introduction

Heart disease is the leading cause of death worldwide (1). The common underlying cause of heart disease is coronary artery disease (CAD). Estrogen is a potential cardio protectant (2) that can be used to treat heart disease in women (3). Although estrogen is specific for women, it is also present at low levels in men (4) and evidence indicates that abnormally high estrogen levels may contribute to cardiovascular disease in men (5).

Recent animal studies indicated that testosterone deficiency hastens atheroma and replacement can prevent this (6); moreover, human trials have shown that atheroma progression was increased in men with lower testosterone, the (7, 8). However, effects of testosterone and estrogen different according to the clinical context for which therapy is used. In a prospective cohort study in men designed to assess the relationship between circulating sex hormones and coronary heart disease, an association was found between increased estradiol level and reduced risk of heart disease (9, 10) .

Our aim in this study was to determine testosterone and estrogen levels in elderly men undergoing angiography due to heart attacks.

Materials and methods

Study participants

Study subjects included 147 men with cardiovascular disease and 45 healthy males. Coronary artery diseases were confirmed by a cardiologist on patients undergoing angiography. Informed consent was obtained from all participants using protocols approved by the Ethics Committee of Mashhad University of Medical Sciences.

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Anthropometric and biochemical measurements
Anthropometric parameters are listed in Table 2. Blood was collected in 5-ml tubes containing 0.5 ml sodium citrate solution and plasma was isolated. All tubes were stored on ice before and after blood collection. Plasma total free testosterone and estrogen concentrations were measured using commercial ELISA kits (Monobind Inc., USA).

DNA Isolation and Genotyping
Genomic DNA was extracted from blood using kits (Favor Gene Corp, Taiwan) according to the manufacturer's protocol. Genotyping of *ENDOG* S12L (rs 2293969) and L142M (rs 61397314) was performed using RFLP-PCR as described previously. The primer sequences are shown in Table 1. PCR amplification was performed in 20 µl reaction mixtures with 5 ng of DNA. Each reaction tube contained 2 µL of 10X reaction buffer (15 mM Tris-HCl, pH 8.0, 50 mM KCl), 1-2 µL of 25 mM MgCl2 solution, 0.5 µL dNTPs mix (10 mM of each), 1 µL of each forward and reverse primer, 0.25 µL Taq (5 units/µL), 5 µL of template DNA, and consisting of initial denaturation at 96 ºC for 10 min followed by 30 cycles with denaturation at 96 ºC for 30 s, annealing at 65 ºC for 30 s, and extension at 74 ºC for 30 s, followed by a final extension at 74 ºC for 10 min. To confirm amplification, we electrophoresed the product in a 2% agarose gel with 100 bp DNA ladder (Cat. No. PR901644)**.** Ten µL of the PCR product was digested with 1-2 µL of Taq 1 (New England Biolab, UK) restriction enzyme, 2 µL of 10X Taq 1 buffer containing 10 mM Tris-HCl, pH 7.5 at 25 °C, 300 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5 mg/mL BSA, and 50% glycerol, and incubated for one hr at 65 ºC. Digested products (20 µl) were electrophoresed on an 8% polyacrylamide gel with 10 bp DNA ladder, and the products were visualized by silver staining. To verify our genotyping result, products were sequenced by the dideoxy chain-termination method.

Statistical analysis
Statistics were analyzed using SPSS 20 (SPSS Inc. USA). Descriptive statistics including mean, frequency, and standard deviation (SD) were determined for all variables and expressed as means ± SDs for normally-distributed variables or as medians and IQRs for not normally-distributed variables. The student's t-test was used for normally-distributed variables. The Mann–Whitney U test was used for continuous variables if they were not normally distributed. Chi-square or Fisher exact tests were used for categorical variables. Logistic regression analysis was used to calculate association between polymorphisms and CVD. All the analyses were two-sided and statistical significance was set at $P < 0.05$.

Results

Statistics were analyzed with 95% accuracy to compare the association between testosterone and estrogen with cardiovascular diseases, and also other variables that can have a destructive effect including blood pressure, age, and history of cardiac disease. The characteristics of cases and controls are shown in Tables 1 and 2, respectively. The subjects were all men. Their median ages were 60.01 for cases and 58.40 for controls. Both case and control subjects underwent angiography for the first time and were analyzed by a cardiologist. The case subjects were angiography positive, and were scheduled for CABG or other interventions, while the control subjects were healthy and angiography negative. The average testosterone and estrogen concentrations were 4.15 and 35.13 for cases, respectively (Table 1) and 5.31 and 47.62 for controls respectively (Table 2), and the average estrogen concentrations were 35.13 for cases (Table 1) and 47.62 for controls (Table 2). Significantly greater blood pressures in cases indicated the most important risk factor for ischemic heart disease. The relationships between each variable and coronary artery disease were first analyzed with χ^2 and then with regression logistic by a backward method for other three variables. Adjusted amounts with significant P value and related risk factor are shown in Tables 3 and 4.

Table 1. Characteristic of Cases by Descriptive Statistics

	Number	Mean
Statistic	Statistic	Std. Error
CHD	101	1.00

Table 2. Characteristic of Controls by Descriptive Statistics

	Number	Mean
Statistic	Statistic	Std. Error
CHD	45	0
Weight	45	68.29
Height	42	173.10
BP	41	13.7512
Smoking	45	0.53
Testosterone	43	5.31
Estrogen	42	47.62
Age	45	58.40
Blood pressure	41	0.17
Valid N	36	

Table 3. Logistics regression for predicting case status

Variable	CHD Disease		SE	P value (adjusted)	OR (95% CI) (adjusted)
	No	Yes			
Blood pressure				0.19	1.19 (0.92-1.55)
normal	34 (33.7%)	67 (66.3%)	0.13		
high	7 (19.4%)	29 (80.6%)			
Age				0.26	1.02 (0.98-1.06)
<60	27 (34.2%)	52 (65.8%)	0.21		
>=60	18 (26.9%)	49 (73.1%)			
Smoking cigarette					
no	21 (29.6%)	50 (70.4%)	0.46	0.57	1.30 (0.53-3.18)
yes	24 (32%)	51 (68%)			
all	45	101			

Table 4. Multiple regression for perfecting testosterone and estrogen concentrations

Variable	S.E.	Sig.	OR	95% C.I. for EXP (B)	
				Lower	Upper
Testosterone	0.153	0.108	0.782	0.580	1.055
Estrogen	0.016	0.003	0.955	0.926	0.984

Discussion

Our data shows that testosterone and estrogen are lower in CHD patients than in healthy controls Moreover, sex hormone levels are age-dependent. In this study the mean case age was 60.01, indicating that testosterone and estrogen levels decline with age and this can lead to cardiovascular disease. We have demonstrated that estrogen and testosterone deficiency is associated with coronary artery disease that leads to heart attack in men. Many of the CAD patients in this study required cardiac surgery. We can conclude that testosterone and estrogen supplementation not only can prevent bone disease but also CAD.

Considerable evidence indicates a direct relationship between the low estrogen and testosterone levels with coronary artery disease; however, more data is needed on the borderline range in men and their association with cardiovascular effects.

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