Relationship Between Serum Free Testosterone Level and Coronary Artery Disease in Postmenopausal Women

Postmenopozal Kadınlarda Koroner Arter Hastalığı ve Serum Serbest Testosteron Düzeyi Arasındaki İlişki

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ABSTRACT Objective: Testosterone is assumed to be a risk factor for coronary artery disease (CAD). However, studies have demonstrated a beneficial effect of testosterone on myocardial ischaemia in men with CAD. To assess the potential role of testosterone in CAD in postmenopausal women, we investigated the association between free testosterone (FT) level and CAD and relationship between FT and other CAD metabolic risk factors. **Material and Methods:** Eighty female patients undergoing diagnostic coronary angiography were studied. Patients were divided into two groups related to the presence of CAD. CAD was documented in 42 patients (CAD+). In all patients serum level of FT, total cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, triglycerides (TG) were measured. CAD+ group had an unfavourable profile of metabolic CAD risk factors as evidenced by elevated total cholesterol, LDL-cholesterol, TG and lower level of HDL-cholesterol ($p< 0.05$). **Results:** We did not find any significant difference in FT levels between two groups ($p> 0.05$) and any relationship between CAD severity (one-vessel vs. multivessel CAD) and FT. **Conclusion:** The results of our study show that in postmenopausal women FT level is not associated with CAD. There are controversial studies on the role of testosterone and FT levels in predicting CAD. Further studies on larger groups would be needed to investigate this issue.

Key Words: Coronary artery disease; testosterone; postmenopause

ÖZET Amaç: Testosteron koroner arter hastalığı (KAH) için bir risk faktörü olarak kabul edilmektedir. Ancak çalışmalar, KAH olan erkeklerdeki miyokardiyal iskemide testosteronun faydalı etkilerini ortaya koymuştur. Postmenopozal kadınlardaki KAH'da testosteronun olası rolünü değerlendirmek için, serbest testosteron (ST) ile KAH ve ST ile diğer KAH metabolik risk faktörleri arasındaki ilişkiyi ortaya koymayı amaçladık. **Gereç ve Yöntemler:** Çalışmaya tanısal koroner anjiyografi yapılan 80 kadın hasta alındı. Koroner hastalığı varlığı veya yokluğuna göre hastalar 2 gruba ayrıldı. KAH 42 hastada saptandı (KAH+). Tüm hastalarda serum ST, total kolesterol, düşük yoğunluklu lipoprotein (LDL) kolesterol, yüksek yoğunluklu lipoprotein (HDL) kolesterol ve trigliserid (TG) değerleri ölçüldü. KAH+ olan grupta yüksek total ve LDL kolesterol, TG ile düşük HDL kolesterol düzeyleri ile ortaya konduğu şekilde metabolik KAH risk faktörleri profili bozuktu ($p< 0.05$). **Bulgular:** Çalışmamızda ST düzeyleri açısından iki grup arasında istatistiksel anlamlı fark saptamadık ($p> 0.05$). Yine iki grup arasında ST ile KAH ciddiyeti (tek damar-çoklu damar) arasında istatistiksel anlamlı fark bulunmadı ($p> 0.05$). **Sonuç:** Çalışmamızın sonuçları postmenopozal kadınlarda ST düzeyinin KAH ile ilişkisi olmadığını göstermektedir. KAH'ı öngörmede testosteron ve ST düzeyleri üzerine çelişkili çalışmalar vardır. Bu konuda daha geniş gruplarda yapılacak daha ileri çalışmalara ihtiyaç vardır.

Anahtar Kelimeler: Koroner arter hastalığı; testosteron; postmenopozal dönem

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The strikingly lower prevalence of myocardial infarction (MI) in premenopausal women than in men of similar age, the progressive narrowing of that difference with age after menopause, and an inability to explain the difference by known risk factors for MI other than gender suggest an important role for sex hormones in the development of MI.[1-4] Women, even postmenopausal, have lower incidence of CAD than men of a similar age. It led to the hypothesis of proatherogenic effects of masculine sex hormones. Android fat tissue distribution was linked with an increased incidence of CAD. An unfavourable influence of testosterone on lipid metabolism and fibrinolysis has further strengthened the hypothesis of possible association between masculine sex hormones and greater risk of atherosclerosis.[5,6]

On the other hand, there are several studies that have strongly challenged this common belief. Stinger et al described improvement in both ischaemia on electrocardiogram and anginal symptoms in men with CAD treated with testosterone propionate.[7] Rosano et al confirmed these findings in the study with acute testosterone administration in the same patient population.[8] English et al documented that CAD in men was associated with lower level of androgens when compared with controls with normal coronary arteries.[9] In women the existing data regarding the potential role of testosterone as CAD risk factor are even more controversial. Philips et al described that testosterone level was related to the severity of CAD in postmenopausal women.[10] Bernini et al documented that in pre- and postmenopausal women, androgens within physiological range correlated with lower risk of atherosclerosis.[11] The studies that evaluate the relationship between CAD and testosterone levels in women are limited and most of them are without control group.

The present study was designed to assess prospectively whether there is a relationship between plasma FT level and CAD in a group of consecutive postmenopausal women undergoing diagnostic coronary angiography. We also evaluated whether FT correlated with the other CAD metabolic risk factors such as smoking, hyperlipidemia, diabetes, arterial hypertension, and family history of CAD.

MATERIAL AND METHODS

With the approval of the local ethics committee and informed consent of the patients, eighty consecutive female patients undergoing diagnostic coronary angiography were studied. The patients had been referred to the cardiac catheterisation laboratory for evaluation of chest pain syndromes and/or abnormal stress tests. Postmenopausal status was defined as an absence of menstrual period for at least 1 year. Exclusion criteria consisted of: previously documented CAD (including history of MI, unstable angina, any operation or cardiovascular intervention < 3 months before the study), age >70 years, endocrine disorders, were taking estrogen, thyroid hormone, insulin, or any other hormone; were taking digitalis, which has been reported to affect the testosterone levels; or had undergone a hysterectomy and significant valvular heart disease referred for coronary angiography before valvular replacement surgery.[12-14] For all women the following clinical details were recorded: height and weight [to calculate body mass index, (BMI)], waist and hip (waist circumference was measured at the umbilicus; hips were measured at the level of maximal circumference; and waist-to-hip ratio was calculated) concomitant drug therapy, conventional CAD risk factors: smoking, hyperlipidemia, diabetes, arterial hypertension, family history of CAD.

CORONARY ANGIOGRAPHY, BLOOD SAMPLING, AND ASSAY METHODS

Coronary angiography was performed via the femoral artery with preformed catheters, and angiograms were taken by use of the Judkins technique with multiple views.[15] Two independent, experienced cardiologists, unaware of the laboratory results, visually estimated the maximum percent reduction in luminal diameter of coronary arteries. Based on generally applied criteria patients were divided into those with CAD (CAD+) and without CAD (CAD-). Blood samples were withdrawn through the needle inserted into the femoral artery for

angiography. All blood samples were taken before 08:00 and 11:00 h after an overnight fast. The serum was obtained after spinning the blood and stored at -70 °C. All measurements, including total cholesterol, HDL-cholesterol, LDL-cholesterol, TG, FT and fasting blood glucose were performed in all patients. Serum levels of glucose, total cholesterol, TG and HDL-cholesterol were determined by enzymatic tests (Roche Diagnostics, Mannheim, Germany). HDL-cholesterol was determined with a homogenous assay (Roche Diagnostics, Mannheim, Germany). LDL-cholesterol was calculated by the Friedewald formula. Plasma level of FT was measured by RIA. The normal level of FT in women by this method ranges from 0.29-1.73 pg/mL.

STATISTICAL ANALYSIS

All statistical analyses were performed with SPSS version 11. Data are expressed as mean ± S.D., or percentage where appropriate. The unpaired Student's *t*-test was used to compare differences between groups. In the multiple-regression model used to determine the relationship of FT and risk factors for CAD. A P-value of less than 0.05 was considered significant.

RESULTS

Within the 6-month study period, 130 postmenopausal women were referred to our Department for coronary angiography. Fifty patients were excluded from the study due to the following reasons: previously documented CAD; history of myocardial infarction or coronary revascularization, referrals before valvular replacement surgery, endocrine disease, polycystic ovary syndrome, hysterectomy, thyroid disease, taking estrogen, thyroid hormone, and insulin. Finally 80 women met the study criteria and comprised the study population. CAD was documented in 42 patients (CAD+): one-vessel CAD was found in 18 women, 12 had two-vessel disease and 12 multivessel disease.

Baseline characteristics of the two groups are displayed in Table 1. There was no statistically significant difference between two groups for age, duration of menopause, waist circumference, presence of diabetes and hypertension, smoking, family history. Patients had similar waist circumference, BMI and concomitant drug therapy. Waist Hip ratio (WHR) was higher in CAD (+) and the

TABLE 1: Baseline characteristics of women with coronary artery disease (CAD+) and without coronary artery disease (CAD-).

	CAD (+) (n= 42)	CAD (-) (n= 38)	P
Age (year)	65 ± 6	63 ± 8	NS
Duration of menopause (year)	16 ± 7.9	15 ± 8.8	NS
Hypertension	26 (61%)	26(68%)	NS
Diabetes	11(26%)	9(23%)	NS
Smoking	1(2%)	2(5%)	NS
Family history of CAD	8(19%)	10(26%)	NS
Waist-hip ratio	0.86 ± 0.1	0.76 ± 0.1	0.04
Waist circumference (cm)	87.4 ± 11.9	83.2 ± 11.5	NS
Body mass index (kg/m²)	30.2 ± 5.2	28.4 ± 4.8	NS
Drug therapy:			
Beta blockers	30	26	NS
Calcium antagonists	11	13	NS
ACE inhibitors	28	26	NS
Statins	22	17	NS
Aspirin	40	37	NS

CAD+, indicates women with coronary artery disease; CAD-, women without coronary artery disease; n indicates number of subjects. Values are mean ± SD or number of subjects. A P-value of more than 0.05 was considered non-significant (NS).

difference in WHR was statistically significant between two groups ($p< 0.05$). Coronary artery disease metabolic risk factors and free testosterone in both groups are displayed in Table 2. CAD(+) group had higher unfavourable profile of CAD metabolic risk factors as evidenced by: elevated total cholesterol, LDL-cholesterol, TG and lower level of HDL-cholesterol. We did not find any significant difference in FT levels between two groups ($p> 0.05$) and any relationship between CAD severity (one-vessel vs. multivessel CAD) and FT. There was no statistically significant difference between FT levels and risk factors by multiple regression analysis ($p> 0.05$).

Exercise stress test was positive in 21 (55%) patients in CAD(-) group. We also couldn't find statistically significant difference between FT levels and CAD risk factors ($p> 0.05$).

DISCUSSION

The main finding of this study was that postmenopausal women with CAD have similar FT level when compared with women without CAD. FT also did not correlate with CAD risk factors. The role of androgens in atherogenesis remains controversial. Results of the studies in animal models have shown that androgens may exert beneficial influences. Hanke et al evaluated the effect of testosterone on plaque development in the aortic ring of male New Zealand White rabbits. They concluded that testosterone inhibited neointimal plaque growing, and vascular androgen receptors could be involved in this process.[16] Crews and Khalil described that both female sex hormones (estrogen and progesterone) and male sex hormone (testosterone) cause relaxation of coronary arteries of castrated male pigs. 17ß-Oestradiol mainly inhibited Ca^{+} -entry, and testosterone acted via other not well-characterised mechanisms.[17] Yue et al documented that testosterone, in both sexes of New Zealand White rabbits, induced endothelium-independent relaxation in isolated coronary artery and aorta. The mechanism in which testosterone regulates vascular tone may involve potassium channels and potassium conductance.[18] One study performed in humans evaluated the relationship between carotid artery intimal-medial thickness (IMT) and androgens. In this study the androgens level was inversely related to IMT in women, suggesting that in women the physiological level of androgens is correlated with lower risk of atherosclerosis.[19] On the other hand, it was documented that women with polycystic ovaries are more likely to develop CAD. In these women however, androgen levels usually surpass physiological ranges.[20,21] In women the existing data regarding the potential role of testosterone as CAD risk factor are even more controversial. Philips et al described that testosterone level was related to the severity of CAD in postmenopausal women.[10] One could speculate that low levels of testosterone could be the result of increased conversion of androstendione to estrone by enzyme aromatase in fat tissue. That may be the case in obese women. Bernini et al documented that in pre- and postmenopausal women, androgens within physiological range correlated with lower risk of atherosclerosis.[11] Kaczmarek et al reported that women with angiographically documented CAD were found to have significantly de-

TABLE 2: Coronary artery disease metabolic risk factors and free testosterone in both groups.

	CAD (+) (n= 42)	CAD (-) (n= 38)	P
Total cholesterol (mg %)	224 ± 35	180 ± 28	0.01
LDL- cholesterol (mg %)	142 ± 34	119 ± 24	0.04
HDL- cholesterol (mg %)	40 ± 8	65 ± 12	0.01
Triglycerides (mg %)	186 ± 84	145 ± 67	0.04
Free testosterone (pg/mL)	1.36 ± 0.67	1.43 ± 0.7	NS

CAD+, indicates women with coronary artery disease; CAD-, women without coronary artery disease; n, number of subjects. Values are mean ± SD. A P-value of more than 0.05 was considered non-significant (NS).

creased testosterone level and TFI in comparison to those without CAD.[22] In our study, testosterone did not correlate with CAD risk factors. Kaczmarek et al found an inverse relationship between testosterone level and lipoprotein (a) a well-known risk factor for CAD, especially in women.[22] In a recent study with 258 male and 236 female postmenopausal participants with angiographically defined stable CAD, levels of estradiol (E2), progesterone (P) and testosterone were not significantly different from levels in the control group. However, in male study participants the ratio of E2 to P and in the postmenopausal women the E2 to P and E2 to T ratios were significantly lower than in the control subjects.[23] He et al reported that significant differences in sex hormone ratios were detected in both male and postmenopausal female participants with angiographically proven CAD.[23]

CONCLUSION

In conclusion, the results of our study show that FT level is not associated with CAD in postmenopausal women who admitted to our hospital. There are controversial studies on the role of T and FT levels in predicting CAD. Further studies on larger groups would be needed to investigate this issue.

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