DE GRUYTER OPEN

Acta Medica Marisiensis 2016;62(2):217-220 DOI:10.1515/amma-2016-0021

RESEARCH ARTICLE

Correlations of Endogenous Testosterone and DHEA-S in Peripheral Arterial Disease

Nagy Elod*, Kun Imre Zoltan, Kelemen Piroska

University of Medicine and Pharmacy Tirgu Mures, Romania

Background: there is an overt bias between cardiovascular morbidity and mortality in male and female patients. Research of the past decades postulated that this difference could be due to the lipid-lowering effect of male sexual-steroids, that show decreased values in cardiovascular disease. **Methods**: the aim of our study was to determine total serum testosterone and dehydroepiandrosterone sulfate (DHEA-S) on a peripheral arterial disease patient's cohort (n=35), in comparison with a healthy control group, (n=23) and to establish correlations with other biological risk factors like serum lipids, C-reactive protein, plasma fibrinogen, and the ankle-brachial pressure index. **Results**: our results showed that total serum testosterone and DHEA-S were significantly decreased in PAD patients in comparison to the control group. We could not observe any significant correlation with the presence of critical ischemia, the levels of total cholesterol, HDL-cholesterol, triglycerides, lipoprotein (a), C-reactive protein or plasma fibrinogen. **Conclusion**: these results express that low androgen levels could be implicated in the pathogenesis of peripheral arterial disease, but testosterone and DHEA-S are not markers of disease severity. The elucidation of their exact role needs larger, population-based studies.

Keywords: testosterone, DHEA-S, serum lipids, peripheral arterial disease

Received: 19 January 2016 / Accepted: 23 March 2016

Introduction

According to a recent 5-year follow-up study the incidence of peripheral arterial disease is approximately 10.2 ‰ in men and 7.5 ‰ in women [1]. A large-scale, population-based study showed that the hazard ratio of British men-to-women in having peripheral arterial disease is 1.74 (1.65-1.85) [2]. The increasing prevalence of atherosclerosis and its complications coincide with a decline in circulating androgen levels in elderly men. An epidemiological study from the US revealed a 10% prevalence after the sixth decade, especially in non-Hispanic whites [3]. It is a challenging question whether sexual-steroids contribute to the gender-related bias of cardiovascular mortality.

The prospective Rotterdam Study highlighted an association between low-level total or free serum testosterone and atherosclerotic lesions on the aorta [4]. In experimental conditions, androgen depletion is associated with PAD and with an inflammatory phenotype [5].

However, data on correlation of endogenous testosterone with peripheral arterial disease are conflicting, since several other studies failed to show any association, especially in multivariate logistic regression models [6-8].

Taking these data into account, we proposed to investigate the levels of total serum testosterone and DHEA-S in PAD patients with a long-term disease history and the correlations of endogenous androgens with clinical and laboratory parameters.

* Correspondence to: Elod Nagy
E-mail: elod.nagy@umftgm.ro

Methods

Patients

We have measured the total serum testosterone levels of 35 male PAD patients in parallel with 23 healthy male controls. All of the PAD patients presented a disease history longer than 2 years and had been treated with haemorheological drugs, antihypertensives, and in the majority of cases, statins. Control persons were age-matched male patients who visited their family physician by the occasion of National Health Survey. Mean age of the groups was 65.03 ±1.77 years (patients) and 62.71±1.61 years (controls). A written consent was obtained from each patient, and the study was approved by the Ethics Committee of Clinical Emergency Hospital, Târgu-Mureş.

Clinical and laboratory parameters

Diagnosis of PAD has been stated based on the presence of intermittent claudication, on clinical examination and assessment of the ankle-brachial pressure index (ABI). Patients were classified according to the Fontaine stages. Stages III and IV, also characterized by an ankle systolic pressure <50 mmHg or a value <0.4 of the ABI, were considered as critical limb ischemia.

Total serum testosterone has been determined by a commercially available competitive ELISA kit (EIAGEN Total testosterone kit, Adaltis, Italy). According to the manufacturer, inter-assay variability of this assay is 3.9%, intra-assay variability is 6.2%, while sensitivity is 0.01 ng/ml. Significant cross-reactivity (10%) has been detected only with dihydrotestosterone. DHEA-S has been measured in 16 patients and 15 controls applying a competitive ELISA assay (DHEA-S, Adaltis, Italy).

Total serum cholesterol and triglycerides, HDL-cholesterol, C-reactive protein and plasma fibrinogen were measured in all patients by automated enzymatic assays on a COBAS INTEGRA 400 analyzer. Lipoprotein (a) and CRP have been measured by automated immuno-turbidimetry assays on Konelab 30 (Thermo Scientific, Finland). Plasma fibrinogen was determined by the Clauss-method (Fibrinogen Reagent, IMMUNO, Austria).

Statistical analysis

Statistical analysis has been performed with STATISTICA 5.0 (Statsoft, USA). According to the small size of the groups and abnormal distribution for the main parameters-testosterone and DHEA-S, we applied non-parametric statistical tests, the Mann-Whitney U test for between-group comparisons, and the Spearman rank-correlation in correlation analysis. Testosterone and DHEA-S are showed both as median (quartile range)– text, and as mean ± SD- figure. Serum lipid levels were normally distributed, therefore we show the median (quartile range) values. The threshold of statistical significance was stated at $p<0.05$.

Results

Analysis and correlations of total serum testosterone levels

Considering the normal range of total serum testosterone indicated by the manufacturer (1.8-9.0 ng/ml), 10 patients (28.5%) and 2 controls (8.7%) showed pathologically decreased values (difference without significance, p=0.09, Fisher exact test). Comparing the testosterone levels in the two groups, we observed lower values in patients – medians and quartile range 4.15 (2.92-5.61) ng/mL vs. 2.62 (1.14-3.59) ng/mL, p=0.019, as it is shown in Figure no.1.

In the overall and in the PAD group, serum testosterone levels showed a weak, but significant decreasing trend with age (R=-0.33, p=0.01, R=-0.33, p=0.04). Testosterone levels showed a slight, but non-significant decrease in advanced vs. early disease. Total testosterone in patients with early-stage (n=22) disease was 2.88 (2.35-2.58) ng/ml, while the subgroup with critical ischaemia (n=13) had 2.47 (2.35-2.58) ng/ml (p=0.82, Mann-Whitney U test).

Testosterone levels in the PAD group have been correlated with other biological parameters and the ankle-brachial pressure index (ABI), a functional measure of disease severity. No significant correlations between testosterone, DHEA-S, serum lipids, CRP, fibrinogen or ABI results were observed, as it is shown in Table I.

DHEA-S levels were also significantly lower in PAD patients: 0.54 (0.46-0.96) μg/ml vs. 1.12 (1.05-2.74) μg/ml compared to controls (medians with quartile range, $p<0.001$) (Figure 1). We could not show a significant influence of critical ischaemia on serum DHEA-S levels. If DHEA-S of the whole group (patients+controls) has been correlated to serum lipids, the correlation was significant

Table I. Correlations of serum testosterone levels with other biological and functional parameters.

Correlation	Spearman R	p-level
Testosterone & DHEA-S*	0.215	0.425
Testosterone & Total cholesterole	0.033	0.866
Testosterone & Tryglicerids	-0.019	0.924
Testosterone & HDL-cholesterole	0.025	0.903
Testosterone & Fibrinogen	-0.105	0.609
Testosterone & hsCRP	0.061	0.746
Testosterone & ABI mean	0.152	0.432
Testosterone & ABI low	0.096	0.619
Testosterone & Lp (a)	0.167	0.436

*calculated on 16 cases

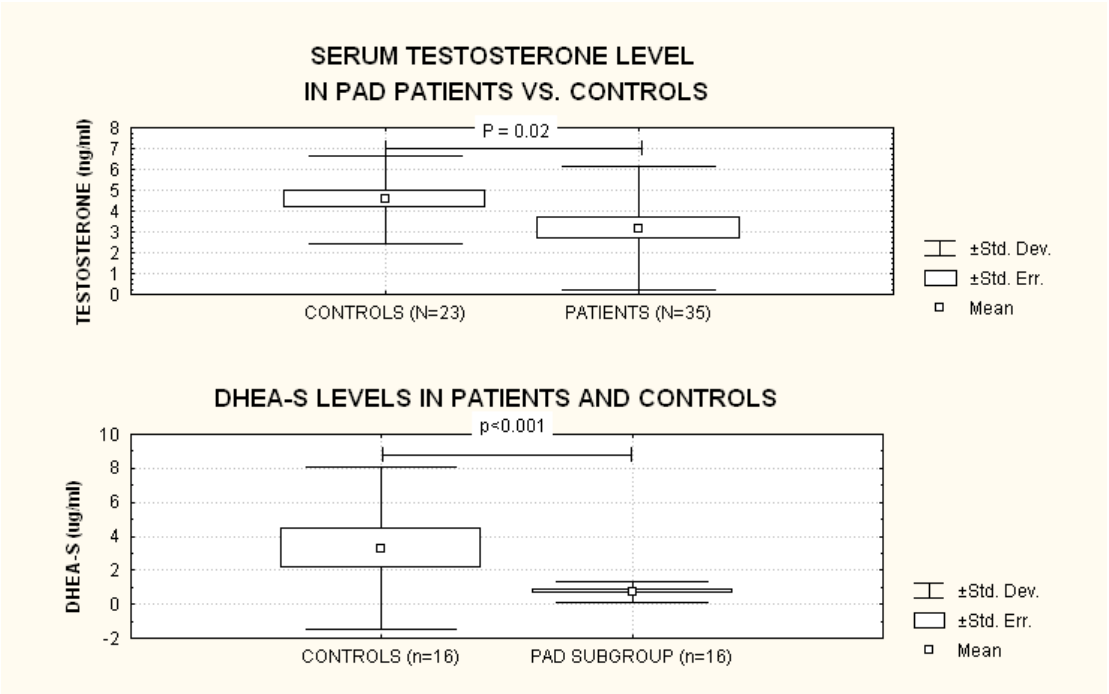

Fig. 1. Box-plot representation of serum total testosterone and DHEA-S levels in PAD patients and controls.

Table II. Comparison of the serum lipid values between the PAD and control group

Lipid parameters	PAD group (n=35)	Controls (n=23)	p value
Total cholesterol (mg%)	178.96 ± 10.44	210.26 ± 16.02	0.11
Tryglicerids (mg%)	174.81 ± 17.01	146.82 ± 10.44	0.18
HDL-cholesterol (mg%)	46.94 ± 2.34	42.23 ± 2.14	0.74
Lipoprotein (a) (mg/l)	454.08 ± 61.50	326.81 ± 66.09	0.23

Parameters given as mean ± SE, p values given for Mann-Whitney U test

for total cholesterol (R=0.35, p=0.02), but not for triglycerides (R=0.28, p=0.08).

Analysis of lipid parameters

24 of 35 patients (68.5%) and only 3 of 23 controls (13%) received long-term statin medication ($p<0.001$, χ^2 test). The values of total serum cholesterol, HDL-cholesterol and serum triglycerides were lower in patients than in controls (Table II.). No significant correlations of the serum testosterone and lipids could be highlighted. Lipoprotein (a) values were apparently higher in patients, but the difference did not reach the threshold of statistical significance (p=0.23, Mann-Whitney U test).

Discussion

There is growing evidence that circulating testosterone is decreased in PAD patients [9]. Our patient group showed significantly decreased total testosterone level than controls, and almost one third of them had levels below the age-adjusted normal reference range. DHEA-S also presented remarkably low values in the PAD group in comparison with healthy controls. Neither total serum testosterone, nor DHEA-S were influenced by the presence of critical ischemia or disease stage.

Testosterone is implicated in regulation of lipid metabolism and of the inflammatory pathway. Sex-hormone binding globulin and total testosterone levels negatively correlate with serum triglycerides, but positively with HDL-cholesterol [10]. However, the exact relationship between androgens and serum lipids is not completely elucidated.

Among the male participants of the Rotterdam Study, statin users possessed lower levels of total serum testosterone and bioavailable, non SHBG-bound testosterone than non-users [11]. Malkin and co-workers found that testosterone replacement therapy lowers the inflammatory cytokine TNFα and IL-1 levels, along with total serum cholesterol in hypogonadal men [12].

One plausible reason for a higher cardiovascular risk in hypotestosteronemic individuals is a biphasic biochemical effect: physiological concentrations of testosterone increase tissue plasminogen activator (tPA) and TFPI mRNA and protein levels, however, overdoses cause a reduction of tPA and TFPI. On the other hand, PAI-1 antigen levels vary reversely with circulating testosterone [13]. Testosterone therapy appears to modulate vascular reactivity and exerts beneficial effects on coronary vascular tone [14].

However, a literature review assesses that short-term testosterone replacement is not improving important measures like the intermittent claudication or the muscle blood flow in PAD [15].

Our patients showed slightly lower lipid levels than controls. At a first view, this finding might be surprising, but it must be taken into account that our cohort consisted of rather old than de novo PAD cases, and that most of the patients but only 3 of controls received statins in their medication.

Despite of the lipid-lowering therapeutic benefit of androgen substitution, and in contradiction with the findings of Bataille and co-workers in the PRIME study [10], we could not observe any significant correlation of androgens (except DHEA-S for the total group) with serum lipid parameters (total cholesterol, triglycerides or HDL-cholesterol). Moreover, there was no detectable correlation between testosterone and lipoprotein (a), a genetically regulated, modified LDL-particle, which has been described as an independent risk factor for cardiovascular disease. According to these results, and in accordance with other authors we confirmed that low total testosterone level is an independent risk factor of PAD [4,16], but it is not a marker of critical ischaemia and disease severity. However, as we performed measurements on a limited number of cases, larger population-based studies are needed to establish the exact diagnostic and prognostic value of serum total testosterone and DHEA-S in PAD.

Conclusions

Our results show that total serum testosterone levels are decreased in peripheral arterial disease. No significant changes of serum testosterone can be observed in advanced disease stages. Both total testosterone and DHEA-S are independent biological risk factors and do not correlate with CRP, plasma fibrinogen or total serum cholesterol.

Funding

This work was partially financed from a Romanian-Hungarian Cooperative Research Grant, Ro-25/05.

Conflict of interest

The authors hereby declare no conflict of interest.

References

1. Alzamora MT, Forés R, Pera G et al. Incidence of peripheral arterial disease in the ARTPER population cohort after 5 years of follow-up. BMC Cardiovasc Disord. 2016;16(1):8 DOI:10.1186/s12872-015-0170-6
2. George J, Rapsomaniki E, Pujades-Rodriguez M et al. How Does Cardiovascular Disease First Present in Women and Men? Incidence of 12 Cardiovascular Diseases in a Contemporary Cohort of 1 937 360 People. Circulation. 2015;132:1320-1328.

3. Criqui MH, Aboyans V. Epidemiology of Peripheral Artery Disease. Circ Res. 2015;116:1509-1526.
4. Hak AE, Witteman JCM, De Jong FH et al. Low levels of Endogenous Androgens Increase the Risk of Atherosclerosis in Elderly Men: The Rotterdam Study. The Journal of Clinical Endocrinology & Metabolism. 2002;87(8):3632-3639.
5. Freeman BM, Mountain DJ, Brock TC et al. Low testosterone elevates interleukin family cytokines in a rodent model: a possible mechanism for the potentiation of vascular disease in androgen-deficient males. J Surg Res. 2014;190(1):319-27.
6. Haring R, Travison TG, Bhasin S et al. Relation between sex hormone concentrations, peripheral arterial disease, and change in ankle-brachial index: findings from the Framingham Heart Study. J Clin Endocrinol Metab. 2011,96(12):3724-32.
7. Kiechl S, Willeit J, Bonora E, Schwarz S, Xu Q. No Association Between Dehydroepiandrosterone Sulfate and Development Atherosclerosis in a Prospective Population Study (Bruneck Study). Arterioscler Thromb Vasc Biol. 2000;20:1094-1100.
8. Price JF, Lee AJ, Fowkes FG. Steroid sex hormones and peripheral arterial disease in the Edinburgh Artery Study. Steroids. 1997;62(12):789-94.
9. Yeap BB, Alfonso H, Chubb SA et al. Lower plasma testosterone or dihydrotestosterone, but not estradiol, is associated with symptoms of intermittent claudication in older men. Clin Endocrinol (Oxf). 2013;79(5):725-32.
10. Bataille V, Perret B, Evans A. et al. Sex hormone-binding globulin is a major determinant of the lipid profile: the PRIME study. Atherosclerosis. 2005;179(2):369-73
11. de Keyser CE, de Lima FV, de Jong FH et al. Use of statins is associated with lower serum total and non-sex hormone-binding globulin-bound testosterone levels in male participants of the Rotterdam Study. Eur J Endocrinol. 2015;173(2):155-65.
12. Malkin CJ, Pugh PJ, Jones RD, Jones TH, Channer KS. Testosterone as a protective factor against atherosclerosis –immunomodulation and influence upon plaque development and stability. Journal of Endocrinology, 2003;178:373–380.
13. Jin H, Lin J, Fu L et al. Physiological testosterone stimulates tissue plasminogen activator and tissue factor pathway inhibitor and inhibits plasminogen activator inhibitor type 1 release in endothelial cells. Biochem. Cell Biol, 2007;85(2):246-251.
14. Jones RD, Jones TH, Channer KS. The influence of testosterone upon vascular reactivity. European Journal of Endocrinology, 2004;151:29-37.
15. Price J, Leng GC. Steroid sex hormones for lower limb atherosclerosis. Cochrane Database Syst Rev. 2012;10:CD000188 doi: 10.1002/14651858.CD000188.pub2
16. Tivesten A, Mellström D, Jutberger H. et al. Low serum testosterone and high serum estradiol associate with lower extremity peripheral arterial disease in elderly men. The MrOS Study in Sweden. J. Am. Col. Cardiol. 2007;50(11):1070-1076.