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Original Research

The role of testosterone deficiency in men with heart failure as a factor of changes in interaction between markers of inflammation, tissue fibrosis and advanced glycation end products

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Abstract

Aim: The aim was to evaluate interaction between serum level of testosterone with C-reactive protein, matrix metalloproteinases-9 activity, advanced glycation end products, galectin-3 levels in men with heart failure. **Methods:** The study included 45 men with heart failure and benign prostatic hyperplasia. Advanced glycation end products, matrix metalloproteinases-9 activity, galectin-3, C-reactive protein, testosterone level was determined in all of patients. The 1st group was made up of men with heart failure and benign prostatic hyperplasia with testosterone deficiency; 2nd group – of men without testosterone deficiency. **Results:** Men with heart failure and benign prostatic hyperplasia with testosterone deficiency had a significantly higher level of advanced glycation end products, galectin-3, matrix metalloproteinase-9 activity ($p<0.001$). Correlation relations between serum advanced glycation end products in patients of the main group with age, ejection fraction, testosterone level were determined. ROC-analysis results for serum advanced glycation end products have shown the highest degree of sensitivity and specificity ($p<0.001$). **Conclusion:** men with heart failure with preserved ejection fraction with testosterone deficiency are characterized by increased serum advanced glycation end products, markers of fibrosis and inflammation. Serum advanced glycation end products are potential biomarkers of developing heart failure with the phenotype of preserved ejection fraction in men with heart failure and testosterone deficiency.

Keywords: heart failure, testosterone deficiency, advanced glycation end products, matrix metalloproteinases-9 activity, galectin-3.

"what is new/what is important" In the present study, we demonstrated that low testosterone level in non-diabetic middle-aged men with heart failure with preserved ejection fraction and benign prostatic hyperplasia was associated with increasing of serum level of advanced glycation end products galectin-3, C-reactive protein, matrix metalloproteinases-9 activity. Serum level of advanced glycation end products has demonstrated good prognostic characteristics concerning developing heart failure with preserved ejection fraction while galectin-3, C-reactive protein, matrix metalloproteinases-9 activity levels had not enough predictive strength.

Introduction

There has been growing interest in the hormonal disorders that accompany heart failure (HF),thus a deficiency of testosterone has been shown an independent marker for worse outcomes in patients with HF [1, 2, 3]. Several studies

have shown that low testosterone level is an independent risk factor for poor prognosis in males with HF, associated with decreased survival in patients with coronary heart disease [4, 5].

As known, benign prostatic hyperplasia (BPH) has been positioned as a new metabolic disease of the aging male with a high prevalence of cardiovascular comorbidity [18]. Several data indicate that low testosterone, more than high testosterone, might have a negative impact on prostate metabolism [19]. Aging men have an increased risk of developing cardiovascular diseases and BPH [20].

Results of numerous studies have shown decreased testosterone level is associated with development of metabolic syndrome, type 2 diabetes, and cardiovascular disease [6–9]. Further, the low testosterone level was associated with high serum levels of insulin resistance, advanced glycation end products (AGEs) in men without diabetes [10]. Should also be noted tes-tosterone attenuates matrix metalloproteinase activity (MMPs) and the cellular processes of the intima in vitro [11]. Thus the increased level of MMPs activity caused by low testosterone levels may lead to the progression of vascular remodeling.

The development and progression of cardiac fibrosis are associated with low testosterone levels and inflammatory biomarkers [12]. In the same time the specific role of testosterone in pathways of myocardial remodeling with the formation of HF phenotypes remains entirely unclear.

The aim of the present study was to evaluate interaction between serum level of testosterone with C-reactive protein, matrix metalloproteinases-9 activity, advanced glycation end products, galectin-3 levels in men with heart failure.

Materials and methods

Baseline study

The study was conducted with approval from the Local Ethics Committee according to principles outlined in the Helsinki declaration. All participants of the research gave informed written consent. The study included men (n=45) aged 45–75 years with HF according to ESC guidelines [13] and estimated diagnosis of BPH according to EAU guidelines [14]. Patients with acute myocardial infarction (<6 months), past Q-myocardial infarction, stable angina with functional class 4, diabetes mellitus (DM), kidney insufficiency, hepatic failure, and prostate cancer were excluded.

Standard laboratory blood tests for erythrocyte sedimentation rate, CRP, hematological parameters, lipid profile, glucose, renal, and liver function tests were performed for all patients. In order to evaluate the state of androgen deficiency the testosterone level was determined by the method of immunoenzymatic analysis with the reagent test kit "AccuBind ELISA".

The fluorescent AGEs in plasma were analyzed by quantitative autofluorescence (fluorimeter Hoefer DQ 2000, USA) with the fixed spectrum of excitation at 460 nm with 20% quinine solution as a standard with results expressed with conversion to glycated albumin.

The MMP-9 activity was analyzed by separating serum proteins (100 µg/track) on 7.5% SDS-PAGE gels copolymerized with gelatin (3 mg/ml). After electrophoresis, the gels were washed twice for 30 minutes in gold 2.5% (v/v) Triton X-100 to remove SDS, and then 5 times for 5 minutes in cold bidistilled water. After washing, gels were incubated overnight at 37°C in developing 50 mM tris-HCl buffer (pH 7.6), containing 0.15 M NaCl, 5 mM $CaCl_2$, 1 mM $ZnCl_2$, and 0.02% Tween-80. The zymograms were visualized and analyzed densitometrically. The level of galectin-3, CRP was determined by the method of immunoenzymatic analysis with the reagent test kit "AccuBind ELISA".

We divided all included patients into two main groups: 1st group with 24 men with HF and BPH with testosterone deficiency (testosterone level <2.5 ng/ml); 2nd group was made by 21 men with HF and BPH without testosterone deficiency.

The control group consisted of 35 healthy men without HF and normal testosterone level.

Clinical characteristics of patients were summarized in Table 1.

Statistical processing of the obtained results was performed using the licensed program STATISTICS. Non-parametric statistics

were used. The data was presented in the form of a median (Me) and the interquartile segment [25%; 75%]. Continuous data were described as median (interquartile range) and compared with U Mann-Whitney test. Categorical data were described as n (valid%) with account for missing data and compared using Fisher's exact test. For comparison of indicators in two independent groups, the Mann-Whitney U-test. The Spearman rank-order correlation analysis was performed. We used the Receiver Operating Characteristic (ROC) curve to evaluate the potential of AGEs, galectin-3, CRP, and MMP-9 as putative biomarkers for the evolution of the clinical forms of HF. Statistically significant differences in research results were determined at a level of $p < 0.001$.

Results

The level of AGEs in patients with HF and BPH with testosterone deficiency ranged from 0.11–0.19 a.u. / ml, the median was 0.16 [0.07; 0.16] a.u. / ml, in the control group 0.08 [0.04; 0.10] a.u. / ml, respectively (p=0.0001). Increased level of AGEs was established in 24 (53.3%) patients of the main group and 13 (37.1%) of controls (p<0.001). It was found that men with HFpEF and BPH with testosterone deficiency had a significantly higher level of AGEs in comparison with men with HF with reduced EF (HFrEF) with testosterone deficiency and controls (p<0.001) (Table 2). Correlation relations between serum AGEs in patients of the main group with age, EF, testosterone level were determined – R=0.49 (p<0.001), R=-0.60 (p<0.001), R=-0.68 (p<0.001), respectively.

Increased level of galectin-3 was established in 31 (68.9%) patients of the main group and 14 (40%) of controls (p<0.001). The median galectin-3 level in patients with HF and BPH with testosterone deficiency was 6.5 [6.2; 9.7] ng/ml, in the control group 5.2 [4.2; 6.4] ng/ml (p<0.001). A significant difference was established between 1st, 2nd patients group and the control group (p<0.001) (Table 2). The galectin-3 level correlated with age (R=0.72, p<0.001), serum AGEs level (R=0.58, p<0.001), GFR (R=-0.45, p<0.001).

Table 1: Baseline characteristics of the study patients.

Characteristics of the patients	Study patients (n=45)	Control group (n=35)	p-Value
Median of age (years)	65 [56.1; 72.7]	64 [53.8; 70.5]	>0.001
Median level of left ventricle ejection fraction (%)	63 [52; 69]	62 [52.3; 67.8]	>0.001
Glomerular filtration rate (GFR), (ml/min 1.73 m^2)	75 [66; 78]	78 [64; 80.2]	>0.001
Systolic blood pressure (mm Hg)	135.5 [125.0; 145.4]	133.7 [125.4; 144.3]	>0.001
Diastolic blood pressure (mm Hg)	77.2 [70.5; 76.13]	73.5 [71.2; 77.4]	>0.001
Heart rate (per minute)	69.8 [62.5; 74.7]	67.7 [62.0; 74.7]	>0.001
Functional class (FC) (NYHA), %: II III	62 33	62 39	>0.001 >0.001
Patients, received cardiology treatment (%):	82	77	>0.001
ACE inhibitors/ACE receptors	77	73	>0.001
blockers	82	76	>0.001
aldosterone antagonists	74	76	>0.001
β-blockers	69	62	>0.001
calcium antagonists	56	54	>0.001
statins	77	70	>0.001
antiplatelet agents	74	72	>0.001
diuretics	77	74	>0.001

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Table 2: Serum levels of AGEs, Galectin-3 in men with HF and BPH with testosterone deficiency depending on EF.

Indicators	1st group HFrEF and testosterone deficiency (n=24)	2nd group HFpEF and testosterone deficiency (n=21)	Control 1 HFrEF without testosterone deficiency (n=18)	Control 2 HFpEF without testosterone deficiency (n=15)
Advanced glycated end products (AGE) (a.u. / ml)	0.11 [0.08; 0.13]	0.16 [0.13; 0.22]*	0.09 [0.07; 0.11]@	0.09 [0.08; 0.12]@
Galectin-3 (ng/ml)	5.2 [3.9; 7.8]	7.3 [6.8; 9.1]*	4.6 [3.1; 6.5]	5.1 [4.0; 6.4]@

*p<0.001 between 1 and 2 groups. @p<0.001 between 1 and 2 groups and control 1, 2, respectively.

The level of MMP-9 activity in patients with HF and BPH with testosterone deficiency ranged from 11.0 to 18.8 relative units, the median was 215.7 [165.9; 225.7] a.u., in the control group 178.2 [154.8; 186.1] a.u., respectively (p<0.001). It was established significant differences between study groups in activity MMP-9 level (Table 3). Particularly, HFrEF patients with testosterone deficiency had significantly higher MMP-9 activity levels on 17.6% than HFpEF men with BPH and testosterone deficiency, on 26.4% than HFpEF men without testosterone deficiency (p<0.001). Correlation relations between MMP-9 activity level in patients of the main group were determined with ejection fraction, serum AGEs level, testosterone level – R=0.55 (p<0.001), R=0.62 (p<0.001), R=0.68 (p<0.001), respectively.

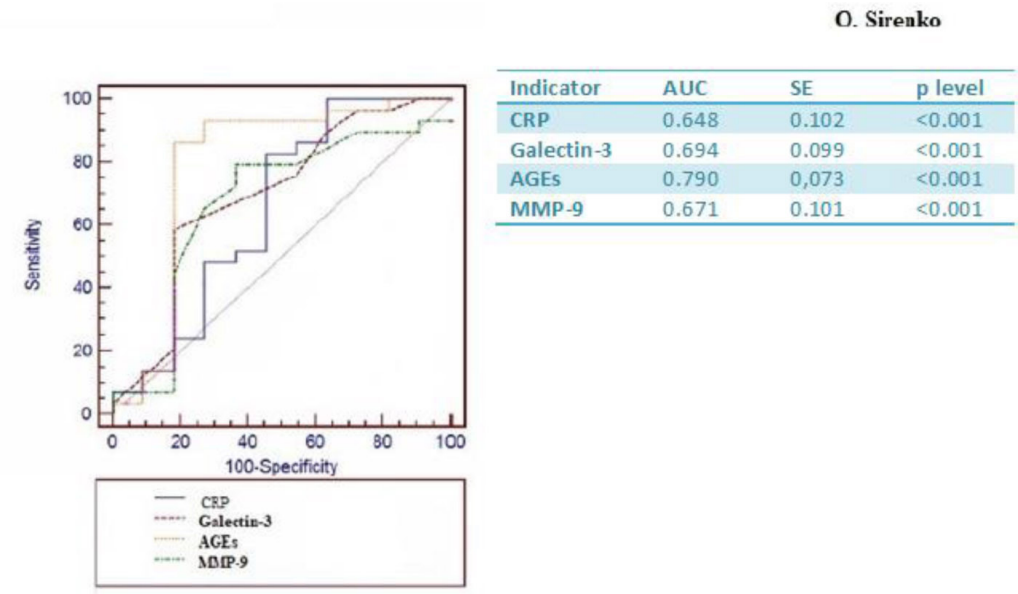
The median CRP level in men with HF and BPH with testosterone deficiency was 6.5 [4.7; 7.2] mmol/l, in the control group 5.7 [3.6; 6.2] mmol/l (p<0.001). A significant difference was established between 1st patients group and the control 1 group (p<0.001) (Table 3). HFrEF patients with testosterone deficiency had significantly higher CRP levels of 23.3% than HFrEF men without testosterone deficiency (p<0.001). The CRP level correlated with MMP-9 activity level (R=0.68, p<0.001), serum AGEs level (R=0.66, p<0.001), GFR (R=-0.56, p<0.001).

Our data show that CRP does not have enough sensitivity and specificity to segregate HFpEF from the HFrEF in men with HF and BPH with testosterone deficiency (Figure 1). Nevertheless, differences in AGEs, MMP-9, galectin-3 levels were statistically significant and able to differentiate HFpEF from HFrEF in patients with testosterone deficiency (p<0.001). It should be noted that only ROC-analysis results for serum advanced glycation end products have shown a high degree of sensitivity and specificity (Figure 1).

Discussion

Trials for HFpEF have not shown clear reductions in morbidity and mortality; however, patients often have other medical conditions that are therapeutic targets. Although guidelines indicate that the treatment of HFrEF and HFpEF should be very different, in clinical practice, and there are no isolated guidelines on the management of HFpEF [13].

Many patients with HFpEF are generally older and have multiple comorbidities including androgen deficiency. The study by Chung et al. explores the possible cardiac mechanism of testosterone a modulatory role in cardiac fibrosis [15]. The authors reported no change in baseline cardiac fibroblast proliferative and migration potential but described an androgen receptor-mediated antiproliferative, anti-collagen, and anti-fibrotic effect of physiological testosterone levels in the myocardium unaffected by a pathological process. Although they recognized that the cardiac fibroblasts were from normal hearts, the authors' conclusion was that testosterone decreased the production of collagen after transforming growth factor-β1 and angiotensin II stimulation which can attenuate the genesis of



Indicator	AUC	SE	p level
CRP	0.648	0.102	<0.001
Galectin-3	0.694	0.099	<0.001
AGEs	0.790	0,073	<0.001
MMP-9	0.671	0.101	<0.001

Figure 1: ROC curve analysis for evaluation of prognostic role of biomarkers for develop of HFpEF in men with HF and BPH with testosterone deficiency.

cardiac fibrosis under pathological conditions. However, in pathological conditions testosterone effects could be quite the opposite. There is a possibility that normal testosterone levels within a physiological range have beneficial biological effects only in relatively healthy individuals from the cardiovascular point of view. Although supplementation of testosterone in HF patients should be considered as potentially increasing the risk of cancer.

In the present study, we demonstrated that besides metabolic risk factors serum levels of AGEs were correlated with low testosterone levels in non-diabetic men with HFpEF and BPH. Besides, serum level of AGEs has demonstrated good prognostic characteristics concerning developing HFpEF while galectin-3, CRP, MMP-9 activity level had not enough predictive strength. There is accumulating evidence that AGEs play a role in the development and progression of cardiovascular diseases in both animal models and humans [16]. In addition, Kilhovd et al. reported that serum levels of AGEs could predict total, cardiovascular disease and coronary heart disease mortality in non-diabetic subjects, especially women [17]. These observations suggest that high circulating levels of AGEs may partly explain the increased risk of future cardiovascular events in men with low testosterone.

In addition, the present research demonstrates in men with BPH testosterone deficiency associated with increasing both fibrosis and inflammation markers that correlate with the intensive glycation process. Certainly, further research in this area is needed.

Limitations

However, the results of this study should be interpreted with caution because of several limitations. Therefore, only men with HF and BPH were chosen for this study. It could be perspectival to evaluate another marker of fibrosis and inflammation, insulin resistance in those patients, especially their dynamics on testosterone supplementation. In order to exclude the confounders that could affect the relationship between serum levels of AGEs, and testosterone, we have chosen our analysis with non-diabetic men and investigating this parameter in the diabetic population needs further research.

Conclusion

The testosterone deficiency in men with heart failure is associated with changes in

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interaction between markers of inflammation, tissue fibrosis and advanced glycation end products. Increased serum AGEs could be potential biomarkers of developing heart failure with preserved ejection fraction in middle-aged men with androgen deficiency.

Conflict of interest disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

Financial disclosures

The authors declare that there is no financial arrangements regarding this manuscript.

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