Testosterone and Blood Pressure Levels in Prehypertensive Men in Calabar, Nigeria

A. Agbecha¹ and U. I. Anwana²

¹Department of Chemical Pathology, Federal Medical Center Makurdi, Nigeria. ²Department of Biochemistry, University of Uyo, Nigeria.

Authors’ contributions

This work was carried out in collaboration between both authors. Author AA designed the study and wrote the protocol. Authors AA and UIA wrote the first draft of the manuscript and performed the statistical analysis. Author AA managed the analyses of the study. Authors AA and UIA managed the literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JALSI/2018/41456

Editors:
(1) Shilpa Chadaga, Department of Biochemistry, Sri Siddhartha Medical College, India.

Reviewers:
(1) Dhaastagir Sheriff, Benghazi University, Libya.
(2) Antonione Santos Bezerra Pinto, Federal University of Ceará, Fortaleza, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history/25925

Original Research Article

ABSTRACT

Background: Evidence exists for an association of low testosterone with hypertension in men. However, no data pertaining to testosterone levels in prehypertensive men exist.

Aim: This study aimed at evaluating the relationship between endogenous total testosterone levels and blood pressure (BP) in prehypertensive men.

Methods: The case-control study comprised a total of 60 apparently healthy men aged 40 to 49 years, attending general check up at the university teaching hospital from December 2015 to February 2016. The participants were divided into 30 prehypertensive and 30 normotensive groups. Fasting plasma total testosterone, C-peptide, glucose (FPG), glycated hemoglobin (HbA1c) and homeostasis model assessment of insulin resistance (HOMA-IR) of the prehypertensive men were compared with age and adiposity matched normotensive controls using the student's t-test. Associations of total testosterone with blood pressure (systolic-SBP and diastolic-DBP), testosterone with confounding parameters in prehypertensive and normotensive men were determined using Pearson correlation analysis.

Results: Comparison of biochemical parameters of prehypertensive (SBP: 131.53±3.20; DBP:...
1. INTRODUCTION

Testosterone is classically referred to as the male androgen, which plays a role in sexual function and general well being of the body [1]. Decreases in endogenous testosterone have physiologic consequences; it is associated with the process of ageing in men, which includes effects like erectile dysfunction, mood depression and cognitive impairment [2,3]. Previous case-control and cross-sectional studies reported an inverse association of endogenous testosterone with hypertension and blood pressure [4,5]. Exogenous testosterone supplementation has demonstrated beneficial effects of testosterone on blood pressure [6]. The high prevalence of sexual dysfunction in untreated hypertensive men could be linked with reduced testosterone [7]. However, prospective studies reported conflicting results, a majority suggested an inverse relationship between endogenous testosterone, hypertension and blood pressure [8,9], while others reported no association [10]. Low testosterone levels have also been associated with increased risk of cardiovascular diseases [11].

Three different categories of blood pressure have been described by the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment (JNC 7) of high blood pressure [12]. These include; normotension when the systolic and diastolic blood pressure (BP) is respectively <120 and <80 mm Hg; prehypertension when systolic BP is 120 to 139 mm Hg or diastolic BP is 80 to 89 mm Hg; and hypertension if systolic BP is ≥140 or diastolic BP is >90 mm Hg. Prehypertension frequently progresses to clinical hypertension over several years especially in older patients [13]. Elevated blood pressure and hypertension are associated with an increased risk of major cardiovascular events including heart diseases and stroke [12].

There is no previous data pertaining to endogenous testosterone levels in prehypertensive men. Our study aimed at determining the association of endogenous testosterone with blood pressure and comparing testosterone levels of prehypertensive men, with those of anthropometrically matched normotensive men.

2. MATERIALS AND METHODS

2.1 Study Design

This case-control study involved the comparison of anthropometric and biochemical parameters of prehypertensives with normotensive controls.

2.2 Study Area and Population

The study was carried out at the University of Calabar teaching hospital, Nigeria. The population comprised male patients aged 40 to 49 years, attending general check-up at the hospital from December 2015 to February 2016.

2.3 Sample Size and Sampling Technique

The sample size was determined using the formula for case-control studies that compare two group means [14]; \( n = 1 + 2C(s/d)^2 \), where \( s \) is the standard deviation (an estimate of the population standard deviation of blood pressure), \( d \) is the effect size (the estimated mean difference of the blood pressure of prehypertensives and normotensives) and \( C \) is a constant dependent on the level of significance and statistical power. At a significance level of

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<tr>
<td>SBP</td>
<td>116.87±6.03</td>
</tr>
<tr>
<td>DBP</td>
<td>78.63±6.95</td>
</tr>
<tr>
<td>Prehypertensive men</td>
<td>85.63±9.09</td>
</tr>
<tr>
<td>Normotensive controls</td>
<td>4.29±1.34</td>
</tr>
</tbody>
</table>
| Pearson's correlation analysis revealed a significant inverse (\( r = -0.577; P = 0.001 \)) correlation between testosterone and age in normotensive controls, whereas no significant inverse correlation (\( r = -0.329; P = 0.076 \)) was observed between testosterone and age in prehypertensive men. A significant inverse correlation was observed between testosterone and SBP (\( r = -0.423; P = 0.02 \)) and testosterone and DBP (\( r = 0.377; P = 0.04 \)) in prehypertensive men. However, testosterone levels in normotensives showed no significant (\( P > 0.05 \)) correlation with blood pressure. No significant (\( P > 0.05 \)) correlation was found between testosterone and metabolic indices in the study groups.

Conclusions: This highly selected population of middle-aged prehypertensive men demonstrates decreased endogenous total testosterone levels with increasing blood pressure.

Keywords: Testosterone; prehypertension; normotension; blood pressure.
5%, and statistical power of 90%, C is 10.51. The calculated sample size was 28.6, which was approximated to 30 for each group. The male participants were randomly assigned to prehypertensive and normotensive groups.

2.4 Selection Criteria

After obtaining ethical clearance from the institutional ethics committee and a written informed consent from each participant, information regarding their bio-data, detailed medical history, dietary, lifestyle and physical activity was obtained. All other body parameters were evaluated in the morning, between 8:00 AM and 9:00 AM.

2.4.1 Inclusion criteria

The inclusion criteria were the following: men within the ages of 40 and 49 years, with no history of hypertension, not on antihypertensives, no history of cardiovascular disease (coronary disease, stroke, peripheral vascular disease) and BMI <28 kg/m².

2.4.2 Exclusion criteria

Patients with diabetes mellitus, smoking habits, history of alcohol abuse (intake of more than 30 mL of ethanol per day), previous sexual dysfunction, and conditions requiring any medication were excluded from the study.

2.5 Data Collection

The clinical evaluation included BP, body weight, height, waist circumference measurements and venous blood samples drawing for the determination of total testosterone, fasting plasma glucose (FPG) and connecting peptide (C-peptide).

2.5.1 Blood pressure

The systolic and diastolic blood pressure was measured after 15 and 25 min of rest in the sitting position using an aneroid manometric sphygmometer, three times with the right arm relaxed and well supported by a table, at an angle of 45-degrees from the trunk.

2.5.2 Body mass index and waist circumference

Body weight to the nearest 0.1 kg and height to the nearest centimeter were measured with the subjects barefoot and in light clothing and BMI was calculated as weight (kilograms)/height (meters squared). Waist circumference (WC) was measured horizontally at the level of the natural waist, which was identified as the level at the hollow molding of the trunk when the trunk was laterally concave.

2.5.3 Blood sample collection

To limit the influence of fluctuations of plasma testosterone levels due to its varied secretion, blood samples for testosterone evaluation were always drawn at the same time of the day, between 8:00 AM and 9:00 AM. All blood samples were collected without venous occlusion into ethylenediaminetetraacetic acid (EDTA), plain and fluoride oxalate vacutainer tubes. Blood in plain and fluoride oxalate vacutainer tubes were centrifuged at 3000 rpm for 10 min within 1 h of blood collection and serum used for the determination of total testosterone, C-peptide while plasma used for fasting glucose determination. EDTA blood was used for the determination of glycated hemoglobin (HbA1c). Fasting plasma glucose was determined immediately after separation; while serum extracted for C-peptide and total testosterone determination was stored at -20°C for laboratory evaluation.

2.6 Laboratory Methods

The Emax microplate reader manufactured by Molecular Devices, Sunnyvale, USA, was used for immunoassay of testosterone and C-peptide, whereas a spectrophotometer OPTIMA SP 300 manufactured by OPTIMA Inc., Tokyo, Japan, was used for analysis of fasting plasma glucose and glycated hemoglobin.

2.6.1 Glycated hemoglobin

Glycated Hemoglobin Kit was obtained from Teico Diagnostics Anaheim California, USA. The determination of HbA1c was based on Trivelli et al. (1971) column chromatographic cation exchange resin method [15].

2.6.2 Glucose

Glucose kit was obtained from Randox Diagnostics Antrim United Kingdom. Barham and Trinder (1972) glucose oxidase method was used in the determination of plasma glucose [16].

2.6.3 Connecting peptide

Connecting peptide Kit was obtained from Diagnostic Automation Inc. Calabasas, USA.
Serum C-peptide was determined by the quantitative solid phase enzyme-linked immunosorbent assay (ELISA) method.

2.6.4 Testosterone

Testosterone Kit was obtained from Diagnostic Automation Inc. Calabasas, USA. The ELISA method was used for the quantitative determination of total testosterone.

2.6.4 Insulin resistance

Insulin resistance was determined by a programmed computerized Microsoft excel HOMA 2 calculator, using FPG and serum C-peptide values.

2.7 Statistical Analysis

The statistical package IBM Armonk, New York, United States SPSS version 21 was used in analyzing the data generated. Descriptive statistics were used in determining the mean and standard deviation of the parameters measured. The student's t-test was used in comparing the mean of the parameters in prehypertensive and control groups. Pearson correlation analyses were done to test the association of testosterone and blood pressure, age and metabolic indices measured in prehypertensive and normotensive men. Two-tailed \( P<.05 \) was considered statistically significant.

3. RESULTS

Plasma testosterone levels together with the anthropometric and metabolic indices of the study population are shown in Table 1. The two groups were matched for age, BMI and WC. Plasma testosterone levels were significantly \( (P=.007) \) lower in prehypertensive men than in normotensive controls. Whereas no significant difference \( (P>.05) \) in HOMA-IR, levels of C-peptide, FPG and HbA1c was observed between prehypertensive and normotensive men. In Table 2 Pearson's correlation analysis showed no significant \( (P=.076) \) inverse correlation \( (r=-.329) \) between testosterone levels and age in prehypertensives. Whereas in Table 3 a significant correlation was observed between testosterone and age in normotensives. In prehypertensive men a significant inverse correlation was found between testosterone levels and systolic BP \( (r=-.423, \ P=.02) \) [Fig 1]; testosterone and diastolic BP \( (r=-.377, \ P=.04) \) [Fig. 2]. Whereas a non-significant correlation \( (P>.05) \) was observed between testosterone and BP values in normotensive men [Table 3]. There was no significant correlation \( (P>.05) \) between testosterone and BMI, WC, FPG, C-peptide, HOMA-IR, HbA1c in both prehypertensive and normotensive men [Tables 2 and 3].

4. DISCUSSION

Few and contrasting findings have been reported in epidemiological trials regarding the relationship between endogenous testosterone and hypertension; some studies have shown reduced testosterone levels in subjects with essential hypertension as compared to normotensive subjects, while other studies have not demonstrated a significant difference in this respect. The disparity among various reports might be due to the following factors: Differences in the characteristics of the examined populations; differences in the methods used for the measurement of testosterone and the selection of study groups.
populations; differences in the methodologies used; and the lack of data control for age, body composition (adiposity), type 2 diabetes, smoking habits, drug intake and history of previous sexual dysfunction, all of which may represent confounding factors. Age, diabetes, and obesity in our study population were taken into account during evaluation of endogenous testosterone since testosterone is known to decline with increasing age, obesity, and diabetes. [17,18]. Also recruiting subjects never placed on antihypertensives, not taking any other drug, not smoking, not abusing alcohol, and without previous sexual dysfunction allowed us to assess the level of testosterone more likely related to prehypertension itself and not to other confounding factors. In an effort to eliminate these confounding effects, we arrived at a small sample size to obtain a homogenous sample that will enhance bias-free statistical decisions.

Table 2. Pearson’s correlation coefficients of testosterone with age and metabolic indices in prehypertensive men

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Testosterone</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.329</td>
<td>0.076</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.117</td>
<td>0.538</td>
</tr>
<tr>
<td>WC</td>
<td>-0.328</td>
<td>0.077</td>
</tr>
<tr>
<td>C-peptide</td>
<td>0.096</td>
<td>0.613</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.071</td>
<td>0.710</td>
</tr>
<tr>
<td>FPG</td>
<td>-0.223</td>
<td>0.235</td>
</tr>
<tr>
<td>HbA1c</td>
<td>-0.023</td>
<td>0.905</td>
</tr>
</tbody>
</table>

WC- waist circumference, BMI- body mass index, FPG- fasting plasma glucose, HOMA-IR- homeostasis model assessment of insulin resistance, C-peptide- connecting peptide, HbA1c- glycated hemoglobin

Results of this study showed low testosterone in pre-hypertensive men, compared with normotensive controls. Similar to our study, however in hypertensive males, Usoro et al., Svarberg et al. Fogari et al. observed low testosterone levels compared to normotensives [19,11,20]. The risk of incident hypertension is shown to be higher in patients with androgen deficiency [8].

Our study showed an inverse relationship between testosterone levels and BP in prehypertensive men. However, no correlation was observed between testosterone and age, BMI, WC, C-peptide, HOMA-IR in prehypertensive men. This finding was independent of the confounding factors of low testosterone. Earlier studies in hypertensive men have shown an association between low testosterone and blood pressure [4,8]. Some population studies in men have also shown an association between low testosterone level and blood pressure [20,1,21]. Previous observational studies suggest that low levels of endogenous testosterone increase the risk of cardiovascular disease in men [9]. The inverse correlation between testosterone levels and blood pressure observed in our study imply that maintenance of normal testosterone in prehypertensive men could normalize their blood pressure, preventing their progression to full hypertension.

Table 3. Pearson’s correlation coefficients of testosterone with age, blood pressure and metabolic indices in normotensive men

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Testosterone</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.577</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI</td>
<td>-0.054</td>
<td>0.777</td>
</tr>
<tr>
<td>WC</td>
<td>-0.023</td>
<td>0.906</td>
</tr>
<tr>
<td>SBP</td>
<td>0.322</td>
<td>0.083</td>
</tr>
<tr>
<td>DBP</td>
<td>0.152</td>
<td>0.424</td>
</tr>
<tr>
<td>C-peptide</td>
<td>0.133</td>
<td>0.483</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>0.119</td>
<td>0.533</td>
</tr>
<tr>
<td>FPG</td>
<td>-0.251</td>
<td>0.181</td>
</tr>
<tr>
<td>HbA1c</td>
<td>-0.296</td>
<td>0.113</td>
</tr>
</tbody>
</table>

**Correlation is significant at the 0.01 level (2-tailed), WC- waist circumference, BMI- body mass index, SBP- systolic blood pressure, DBP- diastolic blood pressure, FPG - fasting plasma glucose, HOMA-IR- homeostatic model assessment of insulin resistance, C-peptide- connecting peptide, HbA1c- glycated hemoglobin**

Our finding of low plasma testosterone levels in prehypertensive suggests a cause and effect mechanism between blood pressure and endogenous testosterone. This mechanism could be predominantly centered on the functional and structural role of testosterone on the vasculature.

Alterations in vascular tone play a major role in the control of blood pressure and the coronary circulation and thereby the incidence of hypertension and coronary artery disease [22]. Endogenous testosterone stimulates vascular dilation by the induction of endothelial-dependent vascular relaxation, confirmed in both human and animal models [23,24]. Low testosterone thus impacts on the functional and structural changes in the properties of the arterial wall, promoting resistance to blood flow accompanied by high blood pressure [25,26]. Vlachopoulos et al., reported the association of low testosterone with aortic thickness and elevated blood pressure in young men and hypertensive individuals [25]. Testosterone suppresses the expression of
proinflammatory cytokines linked to atherosclerosis and stimulates the production of anti-inflammatory cytokines known to be atheroprotective [27]. Studies have demonstrated the association of low testosterone with the inflammatory process of atherosclerosis which clinically presents with elevated blood pressure [28].

Fig. 1. Correlation of testosterone with systolic blood pressure in prehypertensive men

\[ r = -0.423, n=30, P=.02, BP - blood pressure \]

Fig. 2. Correlation of testosterone with diastolic blood pressure in prehypertensive men

\[ r = -0.377, n=30, P=.04, BP - blood pressure \]
Testosterone production pattern could be affected by the functional changes either directly in the testicular Leydig cell or through changes along the Hypothalamic Pituitary Gonadal axis. Leydig cell testosterone biosynthesis is primarily regulated by pulsatile secretion of pituitary luteinizing hormone under the control of hypothalamic gonadotropins [29]. Compelling evidence exists that Leydig cell steroidogenesis is further modulated locally by circulating hormones, growth factors, and cytokines [29].

A genetic base for a mechanistic link between low testosterone and high blood pressure is reported [30]. The genetic hypothesis is supported by data obtained from natriuretic peptide receptor A (NPR 1) gene-deficient and gene duplicated mutant mouse models. Natriuretic peptide receptor A gene which primarily mediates the hypotensive effects of the atrial natriuretic peptide on the cardiovascular system is also expressed in Leydig cells, stimulating testicular steroidogenesis. An NPR 1 gene-deficient male mouse model is characterized by both high BP and low circulating testosterone levels [30]. In addition, men with a family history of hypertension have been shown to have lower than normal serum testosterone levels [31].

The precise mechanism underlying the relationship between hypertension and low testosterone levels remain unresolved.

5. CONCLUSION

In conclusion, the results of this study, which refer to a highly selected population of middle-aged, prehypertensive men with limited generalizability, demonstrates a relationship between prehypertension and impaired plasma testosterone levels. The nature of this relationship with its physiologic and clinical significance might help us to better understand mechanisms of blood pressure control, which merits further investigation.

CONSENT

The patient’s written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

Ethical approval for this study was obtained from the ethics committee of University of Calabar Teaching Hospital, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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Peer-review history:
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