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Original Research Article

Male sex hormone as a correlate of endothelial function in middle-aged Indian males: a cross-sectional prospective observational study

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ABSTRACT

Background: Data on relationship between serum testosterone and endothelial dysfunction measured by brachial artery flow-mediated dilatation (BAFMD) in Indian subset are scarce. The present study was envisaged to assess the correlation between serum testosterone and endothelial dysfunction measured by BAFMD.
Methods: From October 2013 till September 2014, 92 Indian male patients aged 40-60 years who underwent investigation of flow-mediated dilatation of the brachial artery using ultra sonography were included. The association between serum testosterone and BAFMD percent-measured endothelial dysfunction was examined.
Results: Multivariate regression analysis in 92 Indian male patients (mean age 53.12±6.3 years) revealed that low levels of total serum, serum free and serum bioavailable testosterone were significantly associated with BAFMD% and were independent of age, hypertension, diabetes, body mass index (BMI), current smoking and hyperlipidaemia ($p<0.001$). The total serum, serum free and serum bioavailable testosterone were positively correlated with BAFMD% with Pearson correlation coefficients of $r=0.572$, $r=0.525$ and $r=0.547$, respectively ($p<0.001$).
Conclusions: Low levels of total serum, serum free and serum bioavailable testosterone were significantly associated with BAFMD%-measured endothelial dysfunction, irrespective of cardiovascular risk factors.

Keywords: Brachial artery, Dysfunction, Endothelium, Risk factor, Testosterone

INTRODUCTION

The vascular endothelium serves a pivotal role to control vascular permeability, haemostasis, inflammation, thrombosis and vascular tone.[1,2] Impairment of these regulatory functions leads to endothelial dysfunction, which is commonly manifested by decline in endothelial-dependent vasodilatation. Additionally, endothelial dysfunction is considered as a risk factor at an initial and modulating stage in the aetiology of atherosclerotic cardiovascular disease as well as a significant predictor of adverse cardiovascular events.[2-4] Moreover, endothelial function is usually evaluated by two techniques: a) invasive methods like intracoronary acetylcholine infusions and b) non-invasive methods like BAFMD.[3,5,6] The most frequently used non-invasive physiological technique is BAFMD that indicates endothelium-dependent relaxation of brachial artery owing to increased blood flow. The measurement of brachial artery reactivity is used as risk indicator for cardiovascular disease.[3,7,8] However, to the best of our knowledge, only two investigations in the Japanese and German population have revealed a link between serum testosterone and endothelial dysfunction as evaluated by BAFMD.[9,10] In view of the aforementioned observation, this was the first Indian study which determined correlation of serum testosterone with BAFMD%-measured endothelial dysfunction.

METHODS

This was a cross-sectional, prospective, observational, single-center study carried out in a tertiary health care center in consecutive 92 Indian male patients aged 40-60 years who underwent examination of flow-mediated dilatation of the brachial artery from October 2013 to September 2014. Patients who had a history of coronary artery disease (CAD) and had a history of hypogonadism, prostate cancer treated with antiandrogens, liver or renal impairment, current myocardial infarction, recent infection or who were not willing to give informed consent were all ruled out from the study. Testicular volume was measured in patients with a history of dysfunction, low libido or any sign of hypogonadism. These patients were excluded from the current investigation in order to rule out overt hypogonadism as a cause. All patients provided written informed consent form. The study was performed ethically according to the tenets outlined in the Declaration of Helsinki and was approved by the Institutional Ethics Committee.

Data collection

A predefined proforma was used to collect information on each patient's thorough socio-demographic and clinical characteristics. Smoking habits were stratified into two groups: a) current smokers and b) non-smokers. BMI was calculated as weight in kilograms divided by the square of height in metres as well as weight and height were measured after admission.

Sample collection and laboratory measurement

Blood samples were taken in the morning of vascular measurement following overnight fasting of 8 hour. Enzymatic methods were employed on an automated analyser to measure the blood glucose, serum lipid and creatinine levels. Assessment of serum fasting testosterone was done by automated chemiluminescence method via Siemens Immulite 2000 Xpi machine (Siemens, Munich, Germany) (normal levels for men aged 20-49 years: 270-1030 ng/dl; >50 years: 212-755 ng/dl). An automated electro-chemiluminescence method with Roche E170 modular machine (Roche Diagnostics, Basel, Switzerland) was used to measure serum sex hormone binding globulin (SHBG) and an online calculator was used to compute free and bio-available testosterone levels from serum total testosterone and SHBG.

BAFMD measurement

The investigation of BAFMD was performed in a calm, air-conditioned room with patient in a supine position. A longitudinal portion of the brachial artery was examined using a Philips iE 33 ultrasound machine and a linear array transducer (Philips Medical Systems, Andover, MA, USA). A cuff that was positioned above the transducer site was inflated to supra systolic pressure to induce ischemia in the forearm following baseline treatment and deflated following 5 minutes. The BAFMD was computed as percentage increase in diameter between baseline and maximum that was acquired after cuff deflation by using the following equation,

$$\text{BAFMD\%} = \frac{(peak\ diameter - baseline\ diameter)}{baseline\ diameter} \times 100.$$

Statistical analysis

Categorical data were presented as number and percentages. Continuous data were presented as mean±SD. Pearson's correlation test was performed to investigate the correlation between total serum, serum free and serum bioavailable testosterone levels and BAFMD%. Multivariate regression models were done to investigate if testosterone (total serum, serum free and serum bioavailable) was independently associated with BAFMD%. The model was adjusted for all coronary risk factors (age, BMI, smoking history, hypertension, diabetes mellitus, dyslipidaemia as well as history and treatment of ischemic heart disease). A $p<0.05$ was regarded as statistically significant. Statistical tests were executed by using the SPSS statistical software, version 19 (Statistical Package for the Social Sciences, Inc., Chicago, Illinois, USA).

RESULTS

The average age of 92 Indian male patients was 53.1±6.3 years. The study population had higher systolic (132.1±9.2 mmHg) and diastolic blood pressure (81.9±4.2 mmHg) and exhibited higher proportion of hypertension (60.9%) and obesity (46.7%). The mean BAFMD was 13.9±5.1%. The remaining clinical profile of study patients are illustrated in Table 1.

Table 1: Clinical profile of the study population.

Parameters	Total (n=92)
Age (years)	53.12±6.3
Body mass index (kg/m^2)	25.68±3.7
Hemodynamic and vascular parameters	
Systolic blood pressure (mmHg)	132.09±9.2
Diastolic blood pressure (mmHg)	81.89±4.2

Continued.

Parameters	Total (n=92)
Brachial artery flow-mediated dilatation (%)	13.91±5.1
Clinical presentation	
Smoking	35 (38%)
Obesity	48 (52%)
Hypertension	56 (60.9%)
Diabetes mellitus	43 (46.7%)
Dyslipidaemia	33 (35.9%)
Ischemic heart disease	11 (12%)
Laboratory parameters	
Fasting blood glucose (mg/dl)	124.93±44.3
Post prandial blood sugar (mg/dl)	172.26±77.2
Total cholesterol (mg/dl)	156.46±38.9
Triglyceride (mg/dl)	162.11±86.9
High density lipoprotein (mg/dl)	37.24±7.7
Low density lipoprotein (mg/dl)	99.55±35.4
Total testosterone (ng/dl)	421.82±168.2
Sex hormone binding globulin (nmol/l)	36.17±11.10
Free testosterone (ng/dl)	8.3±3.3
Bioavailable testosterone (ng/dl)	194.6±74.7

Data are presented as n (%) or mean±standard deviation.

Table 2: Regression coefficients between total testosterone, free testosterone, bioavailable testosterone and BAFMD% adjusted for cardiovascular risk factors.

Hormones	Regression coefficient	P value
Total testosterone (ng/dl)	0.382	<0.001
Free testosterone (ng/dl)	0.445	<0.001
Bioavailable testosterone (ng/dl)	0.407	<0.001
§ BAFMD%, percent brachial artery flow-mediated dilatation		

Regression coefficient by multiple regression analyses with BAFMD% as a dependant variable and cardiovascular risk factors and testosterone (total, free and bioavailable) as an independent variable are shown.

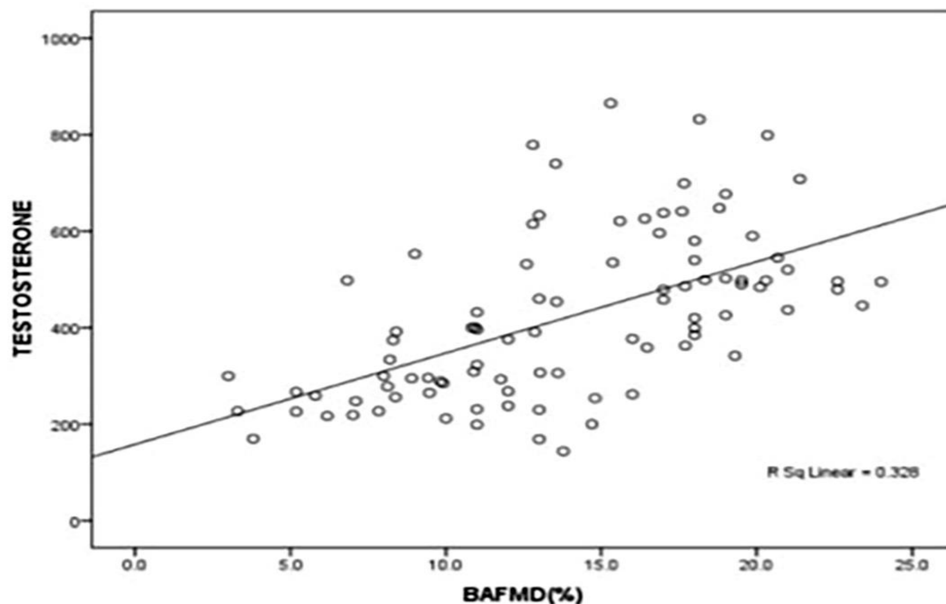

Figure 1: Scatter plot demonstrating correlation between serum total testosterone and BAFMD%.

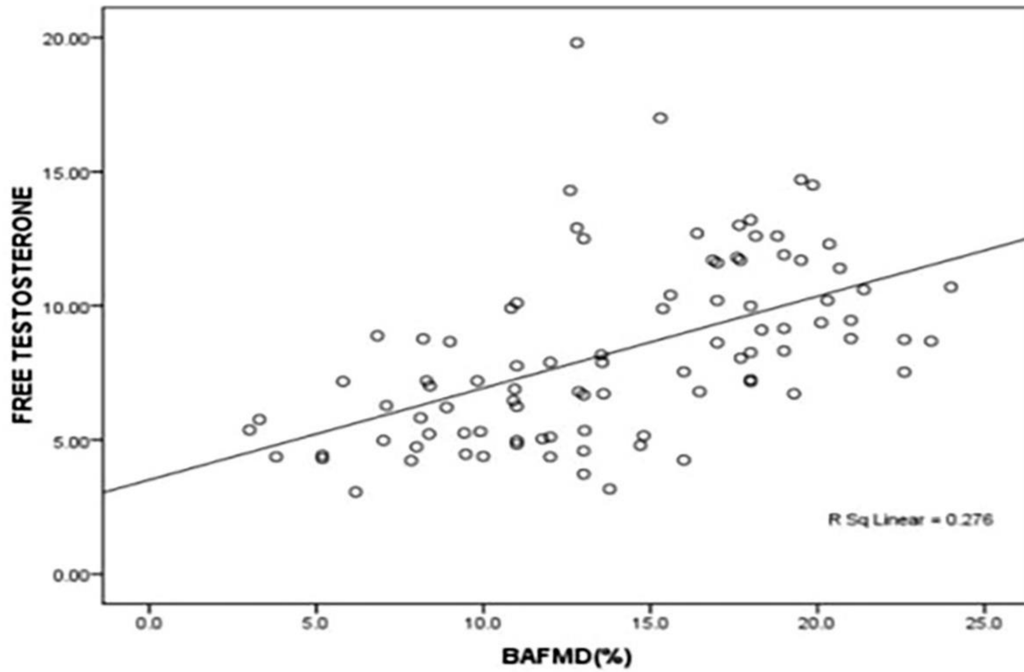

Figure 2: Scatter plot demonstrating correlation between serum free testosterone and BAFMD%.

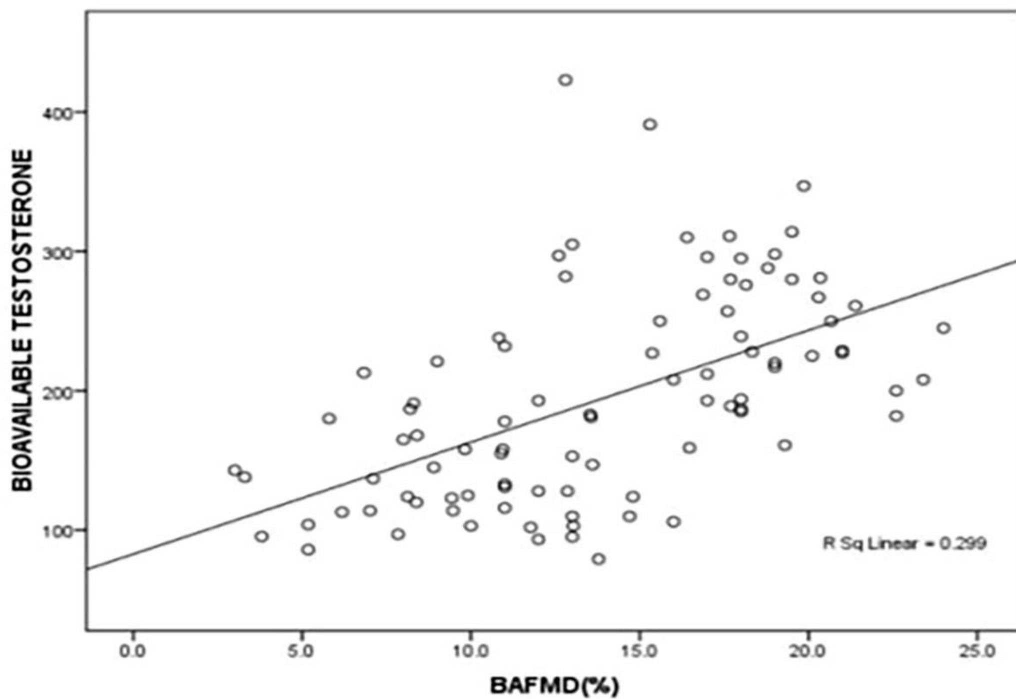

Figure 3: Scatter plot demonstrating correlation between serum bioavailable testosterone and BAFMD%.

Total serum, serum free and serum bioavailable testosterone were significantly correlated with BAFMD% and were unaffected of age, hypertension, diabetes, BMI, current smoking and hyperlipidaemia (p<0.001) as depicted in Table 2. As outlined in Figure 1, total serum testosterone was positively correlated with BAFMD% with Pearson correlation coefficient (r=0.572, p<0.001). There was a considerably significant positive correlation found between serum free testosterone and BAFMD% (r=0.525, p<0.001) (Figure 2). A significant positive

correlation was found between serum bioavailable testosterone and BAFMD% with Pearson correlation coefficient ($r=0.547$, $p<0.001$) as shown in Figure 3.

DISCUSSION

As far as we know, this was the first Indian study that delved into the association of testosterone with BAFMD%-a marker of endothelial function. Because previous research had shown that all conventional risk factors for CAD have a negative impact on endothelial function, thus the present study investigated the link between testosterone levels and endothelial dysfunction evaluated by BAFMD% following adjustment of all coronary risk factors. The current investigation exhibited that low levels of total serum, serum free and serum bioavailable testosterone were correlated with BAFMD%-measured endothelial dysfunction in middle-aged Indian men, even after excluding of all confounding well-known conventional cardiovascular risk factors such as age, hypertension, diabetes, BMI, current smoking and hyperlipidaemia (for total testosterone, $r=0.382$, $p<0.001$; free testosterone, $r=0.445$, $p<0.001$; bioavailable testosterone, $r=0.407$, $p<0.001$). Owing to the aforementioned finding, the investigation demonstrated that the low level of testosterone was a significant predictor of endothelial dysfunction in Indian male patients.

The above mentioned outcome was corroborated by two previous studies including Japanese and German patients.[9,10] In a prospective analysis of 108 consecutive male patients aged 20-79 years, Akshita et al exhibited a correlation between low testosterone levels and decreased flow-mediated dilatation (FMD)% with endothelial dysfunction that was independent of major cardiovascular confounders.[9] This study found a significant positive relationship between testosterone (total and free) and FMD% which was unaffected of age, BMI, hypertension, hyperlipidaemia, diabetes mellitus and smoking ($\beta=0.198$ and 0.247, respectively; $p<0.01$) through multivariate regression analysis. Furthermore, multivariate regression analysis also revealed a significant correlation between total and free testosterone and FMD%, which was unaffected of age, BMI, systolic blood pressure, total cholesterol, high-density lipoprotein cholesterol, fasting plasma glucose, smoking and nitro-glycerine-induced dilatation ($\beta=0.196$ and 0.227, respectively; $p<0.01$). However, the study did not examine the bioavailable testosterone due to the lack of a direct assay to quantify bioavailable testosterone in Japan. Unlike the earlier study, the present investigation had the benefit of measuring all types of testosterones in patients.

Similarly, to the current study, Empen et al in a population-based study observed a significant relationship between low levels of total serum and serum free testosterone with impaired endothelial function in 722 German male patients aged 25-85 years.[10] Multivariate logistic regression analysis revealed that each decrement of total testosterone, free testosterone was significantly associated with decreased FMD after adjustment for potential cardiovascular confounders (for total testosterone: odds ratio-1.30, 95% confidence interval (1.04-1.63): $p=0.023$, free testosterone: odds ratio-1.37, 95% confidence interval (1.06-1.76): $p=0.016$].

Furthermore, prior research on the effect of testosterone levels on endothelial function in males was mostly based on small studies that were either administered anabolic steroids or had a high cardiovascular risk.[9,11-13] Sansone et al in a meta-analysis involving 86 male patients found that acute testosterone administration increased the level of BAFMD.[14] On the other hand, chronic testosterone administration lowered the level of BAFMD. These findings were in accordance with the previous studies.[15,16] However, the results of Sansone et al were not significant.[14] Similarly, various investigators demonstrated that both acute and chronic testosterone administration improved the BAFMD% without affecting the baseline diameter of the vessel.[17,18] Overall, the findings of the present study, which focused on Indian population, were congruent with those of prior studies on other population groups. Further follow up was intended to assess the role of testosterone in endothelial dysfunction measured by BAFMD%.

The present study had few limitations that needed to be addressed. To begin with, it was difficult to establish causal association between serum testosterone and endothelial dysfunction due to cross-sectional design of this study. In addition, because testosterone was evaluated in a single sample, alteration in serum testosterone levels with time could not be determined. Lastly, selection bias cannot be excluded from the study as this was a single-center study with a relatively low sample size, and study patients with or without coronary risk factors.

CONCLUSION

Low total serum, serum free and serum bioavailable testosterone levels were significantly correlated with BAFMD%, irrespective of cardiovascular risk factors and have been identified as independent predictors of endothelial dysfunction. Large prospective, multicentre clinical studies with long-term follow up are warranted to enlighten the specific mechanism behind our findings and its clinical implications.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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