Original Research Article

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

Chandra Mohan¹, Kunal Gururani¹*, Anurag Rawat¹, Mansi Kala²

¹Department of Cardiology, ²Department of Pathology, Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, India

Received: 24 November 2021
Accepted: 10 December 2021

*Correspondence:
Dr. Kunal Gururani,
E-mail: drgururanik@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

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 ultrasoundography 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.¹² Impairment of these regulatory functions leads to endothelial dysfunction, which is commonly manifested by decline in endothelium-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.¹² Moreover, endothelial function is usually evaluated by two techniques: a) invasive methods like intracoronary acetylcholine infusions and b) non-invasive methods like BAFMD.³⁵⁶ 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.³⁷⁸ 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.⁹¹⁰ 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 androgens, 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 hypogonadism, 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,

\[
BAFMD\% = \left(\frac{peak\ diameter - baseline\ diameter}{baseline\ diameter}\right) \times 100.
\]

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.

| Parameters                          | Total (n=92) |
|-------------------------------------|-------------|
| **Age (years)**                     | 53.12±6.3   |
| **Body mass index (kg/m²)**         | 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

| 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

| 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%](image-url)
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 also found between serum bioavailable testosterone and BAFMD% (r=0.519, p<0.001) (Figure 3).

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

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

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Yeboah J, Crouse JR, Hsu FC, Burke GL, Herrington DM. Brachial flow-mediated dilation
predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. Circulation. 2007;115(18):2390-7.
2. Favero G, Paganelli C, Buffoli B, Rodella LF, Rezzani R. Endothelium and its alterations in cardiovascular diseases: life style intervention. Biomed Res Int. 2014;2014:801896.
3. Moreau KL, Babcock MC, Hildreth KL. Sex differences in vascular aging in response to testosterone. Biol Sex Differ. 2020;11(1):1-14.
4. Gururani K, Jose J, George PV. Testosterone as a marker of coronary artery disease severity in middle aged males. Indian Heart J. 2016;68:16-20.
5. Peretz A, Leotta DF, Sullivan JH, Trenga CA, Sands FN, Aulet MR, et al. Flow mediated dilation of the brachial artery: an investigation of methods requiring further standardization. BMC Cardiovasc Disord. 2007;7(1):1-8.
6. Mil AC. Examining endothelial function in humans in vivo: improving guidelines and exploring novel measures. United Kingdom: Liverpool John Moores University; 2018.
7. Antonopoulos AS, Antoniades C. Mechanisms of testosterone deficiency-related endothelial dysfunction: invited commentary for the Hellenic Journal of Cardiology on: Tsikas et al.”Associations between asymmetric dimethylarginine, nitrite-dependent renal carbonic anhydrase activity and plasma testosterone levels in hypogonadal men”. Hellenic J Cardiol. 2018;59(4):207-8.
8. Aversa A, Bruzziches R, Francomano D, Natali M, Gareri P, Spera G. Endothelial dysfunction and erectile dysfunction in the aging man. Int J Urol. 2010;17(1):38-47.
9. Akishita M, Hashimoto M, Ohike Y, Ogawa S, Iijima K, Eto M, et al. Low testosterone level is an independent determinant of endothelial dysfunction in men. Hypertens Res. 2007;30(11):1029-34.
10. Empen K, Lorbeer R, Dörn M, Haring R, Nauck M, Gläser S, et al. Association of testosterone levels with endothelial function in men: results from a population-based study. Arterioscler Thromb Vasc Biol. 2012;32(2):481-6.
11. Sader MA, Griffiths KA, Skilton MR, Wishart SM, Handelsman DJ, Celermajer DS. Physiological testosterone replacement and arterial endothelial function in men. Clin Endocrinol. 2003;59(1):62-7.
12. Ebenbichler C, Sturm W, Gänzer H, Bodner J, Mangweth B, Ritsch A, et al. Flow-mediated, endothelium-dependent vasodilatation is impaired in male body builders taking anabolic-androgenic steroids. Atherosclerosis. 2001;158(2):483-90.
13. Sader MA, Griffiths KA, McCredie RJ, Handelsman DJ, Celermajer DS. Androgenic anabolic steroids and arterial structure and function in male bodybuilders. J Am Coll Cardiol. 2001;37(1):224-30.
14. Sansone A, Rastrelli G, Cignarelli A, Ponce M, Condorelli RA, Giannetta E, et al. Effect of treatment with testosterone on endothelial function in hypogonadal men: a systematic review and meta-analysis. Int J Impot Res. 2020;32(4):379-86.
15. Ong PJ, Patrizi G, Chong WC, Webb CM, Hayward CS, Collins P. Testosterone enhances flow-mediated brachial artery reactivity in men with coronary artery disease. Am J Cardiol. 2000;85(2):269-72.
16. Webb CM, McNeill JG, Hayward CS, DeZeigler D, Collins P. Effects of testosterone on coronary vasomotor regulation in men with coronary heart disease. Circulation. 1999;100(16):1690-6.
17. Dalal J, Low LP, VanPhuoc D, Rahman AR, Reyes E, Soenarta A, et al. The use of medications in the secondary prevention of coronary artery disease in the Asian region. Curr Med Res Opin. 2015;31(3):423-33.
18. Kang SM, Jang Y, Kim JY, Chung N, Cho SY, Chae JS, et al. Effect of oral administration of testosterone on brachial arterial vasoactivity in men with coronary artery disease. Am J Cardiol. 2002;89(7):862-4.