The effect of serum free testosterone level on glycemic control and atherosclerosis in type 2 diabetic men

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Abstract: Background: Atherosclerosis is a complex disease of the arteries characterized by endothelial dysfunction, vascular inflammation, and the build-up of lipids within the intima of the vessel wall. Testosterone has a central or permissive role in pathogenesis of the metabolic syndrome and type 2 diabetes. Insulin resistance is associated with several CVD risk factors such as obesity, dyslipidaemia, hypertension and the proinflammatory state. We aim to disclose the relationship between serum testosterone concentration and carotid atherosclerosis and its risk factors in men with type 2 diabetes. Patients and methods: The study population comprised 123 consecutive men of Type 2 diabetes. Retinopathy and nephropathy were ranked and graded respectively. Cardiovascular disease was defined as the presence of previous myocardial infarction or cerebral infarction. Total cholesterol and triglyceride concentrations were determined and hemoglobin A1c was measured. Assessment for the presence of carotid atherosclerosis was done, using ultrasonographic measurement of carotid intima media thickness (IMT). The relationship between serum testosterone concentration and carotid intima-media thickness IMT was investigated in all patients. Results: The mean of IMT for all patients was 0.96 ± 0.28 mm. Mean IMT was significantly greater in patients with lower concentrations of F-testes than in patients with higher concentrations of F-testes ($P=0.038$). Relationship between serum free testosterone concentration and other variables showed a negative correlation with patients' age, patients' age at onset, duration of diabetes, BMI, HbA_{1c}, systolic and diastolic blood pressure, and total cholesterol concentrations and mean IMT in men with type 2 diabetes. No significant correlation was found between F-testes with triglyceride and negative correlation with mean IMT. Conclusion: Serum free testosterone concentration was found to be low in type 2 diabetic men. It has a negative correlation with patients' age, patients' age at onset of the disease, duration of diabetes, BMI and HbA_{1c}, total cholesterol concentrations systolic and diastolic blood pressure and mean IMT. This may disclose the different mechanisms played by testosterone in the pathogenesis of cardiovascular risk in men with type 2 diabetes.

Keywords: Testosterone, Atherosclerosis, Type 2 Diabetes Mellitus

1. Introduction

Atherosclerosis is a complex disease of the arteries characterized by endothelial dysfunction, vascular inflammation, and the build-up of lipids within the intima of the vessel wall (1). Men develop coronary artery disease and experience coronary events about 10 years earlier than women. Historically, this was always thought to be related to the protective effects of female sex hormones. Testosterone is an important anabolic hormone and has an

anti-insulin effect (2). Testosterone has a central or permissive role in pathogenesis of the metabolic syndrome and type 2 diabetes (3). One of the most important underlying causes of insulin resistance is associated with low testosterone levels to men (4). Studies have reported that men with type 2 diabetes mellitus (T2DM) have a high prevalence of low serum testosterone (5-7). Further, reduced total testosterone (TT) levels have been associated with insulin resistance and subsequent risk for developing T2DM (8, 9).

Insulin resistance is associated with several cardiovascular disease (CVD) risk factors such as obesity, dyslipidaemia, hypertension and the proinflammatory state. Moreover, insulin resistance is one of the major pathophysiological mechanisms leading to abnormalities in glucose tolerance, which further enhances the risk of CVD (10). Insulin elicits metabolism-independent cardioprotection against myocardial ischaemic injury and the development of atherosclerosis, hypertension and heart failure, in which bio-available nitric oxide (NO) plays a significant role as a second messenger of insulin mediated survival signals. Furthermore, interruption of insulin signaling with the endogenous cardiovascular NO system is participatory in the pathogenesis of insulin resistance and its cardiovascular complications (11).

Since the direct association between the testosterone deficiency and cardiovascular risk remains controversial, we aim to disclose the relationship between serum free testosterone concentration and carotid atherosclerosis and its risk factors in men with type 2 diabetes.

2. Patients and Methods

The protocol for the research project was approved by a suitably constituted ethics committee of the hospital, and it is conformed to the provision of the World Medical Association's Declaration of Helsinki. An informed consent has been obtained for all investigations on subjects of the study (and patient anonymity was preserved).

2.1. Study Populations

The study population comprised 123 consecutive men of type 2 diabetes as diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (12). The study was applied from January 2012 to June 2013.

2.2. Clinical Assessment

Retinopathy was ranked as follows: no diabetic retinopathy (NDR), simple diabetic retinopathy (SDR), and proliferative diabetic retinopathy (PDR). Nephropathy was graded as follows: normal albuminuria, urinary albumin excretion (UAE) <30 mg/g creatinine (Cr); microalbuminuria, UAE 30-300 mg/g Cr; or macroalbuminuria, UAE >300 mg/g Cr.

CVD was defined as the presence of previous myocardial infarction or cerebral infarction based on the clinical history or physical examination.

Exclusion Criteria

1. Patients with major organ failure (heart, respiratory, liver, or renal failure).
2. Patients with cancer prostate, prostatectomy, castration or that receiving androgen deprivation therapy.
3. Patients receiving androgens, steroids, or hypolipidemic medications.

4. Patients with hepatitis C virus infection. [because this infection has been reported to cause atherosclerosis (13)].
5. Smoker patients and controls [to avoid the possible interaction between smoking and testosterone serum level (14)].
6. Those with active infection or autoimmune diseases.

2.3. Laboratory Investigations

- Total cholesterol and triglyceride concentrations were determined by standard techniques.
- Hemoglobin HbA_{1c} was measured immunoturbidimetrically (Using COBAS INTEGRA 400 machine; Roche Diagnostics, Indianapolis, Ind).
- Serum free testosterone F-test concentrations were measured in 123 consecutive men with type 2 diabetes using Microparticle Enzyme Immunoassay (MEA) on AX SYM system F-test kit (normal ranges 14.0- 40.0 pg/ml).

2.4. Assessment for the Presence of Carotid Atherosclerosis using Ultrasonographic Measurement of Carotid Intima-Media Thickness (IMT)

B-mode ultrasonographic imaging of the carotid artery was performed using hp SO-NOS 5500 machine with high-resolution, real-time ultrasonography with a 10-MHz transducer. The examination and image analysis were performed by a trained sonographer who remained unaware of other data and whose duplex results have been previously validated. High-resolution B-mode carotid ultrasonography was performed with HDI 5000 ATL machine (Phillips Medical Systems). The subject lay supine with the neck extended and the chin turned contralateral to the side being examined. Scanning involved examination of carotid arteries in transverse and longitudinal planes. The right and left carotid arteries were scanned for measurement of IMT in both the longitudinal and transverse projections over an arterial segment including 30 mm of the distal common carotid artery, the bifurcation, and 15 mm of the internal carotid artery (15). IMT was measured in the far wall of the vessel as the distance from the leading edge of the lumen-intima interface to the leading edge of the intima-adventitia interface. The average measurement was taken as the mean IMT.

The relationship between serum testosterone concentration and carotid intima-media thickness IMT was investigated in all patients. Also, the relationships between serum testosterone concentrations and major cardiovascular risk factors were evaluated, including age, blood pressure, serum lipid profile, and glycemic control (HbA_{1c}), BMI, severity of diabetic retinopathy, severity of diabetic nephropathy, or presence of CVD.

2.5. Statistical Analysis

Data were collected, revised and then analyzed using SPSS version 10.

Quantitative variables were expressed as mean and standard deviation using unpaired- *t* tests for equality of means. Spearman correlation coefficient was used to correlate between serum testosterone and other variables.

A significant statistical was considered with *p* value <0.05.

3. Results

3.1. Study Population

Demographic, clinical and laboratory data of the patients are summarized in Table 1. The study group comprised 123 men type 2 diabetic patients with a mean age of 60.0 ± 7.9 years. Mean age at onset was 49.0 ± 9.7 years with mean disease duration was 11.2 ± 7.1 years. Mean of BMI was 29.11 ± 3.41 , HbA_{1c} (%) was 7.5 ± 1.6 , mean systolic and diastolic blood pressure were 146.70 ± 9.02 and 91.76 ± 9.36 respectively. Laboratory data of the patients' revealed mean total cholesterol of 194.84 ± 33.20 mg/dl (with levels of triglyceride, HDL cholesterol are 151.42 ± 15.84 mg/dl and 38.38 ± 3.07 mg/dl respectively). Numbers of patients with different ranks of Retinopathy and Nephropathy are enumerated. F-tes was found to be 10.6 ± 3.8 pg/ml.

3.2. Carotid Duplex Study and Measurement of IMT Results

The mean of IMT for all patients [right + left/2] was 0.96 ± 0.28 mm. Mean IMT was significantly greater in patients with lower concentrations of F-tes (<10 pg/ml) than in patients with higher concentrations of F-tes (1.03 ± 0.26 vs. 0.88 ± 0.23 mm, *P* = 0.038.) (Table 2)

3.3. Relationships between Serums Free Testosterone Concentration and Other Variables (Table 3)

A negative correlations were found between serum F-tes concentration and age ($r = -0.410$, *p* < 0.0001), age at onset ($r = -0.277$, *p* < 0.000), duration of diabetes ($r = -0.171$, *p* = 0.0104) and BMI ($r = -0.765$, *p* = 0.0001) and HbA_{1c} ($r = -0.119$, *p* = 0.0409). A negative correlations was found between serum F-tes concentration and systolic and diastolic blood pressure ($r = -0.601$, *p* = 0.0001, $r = -0.457$, *p* = 0.001). Moreover, a negative correlation was found between serum F-tes and total cholesterol concentrations ($r = -0.431$, *p* = 0.002). No significant correlation was found between F-test with and triglyceride (*p* > 0.05). Negative correlations was detected between F-tes concentration and mean IMT ($r = -0.196$, *p* 0.0093) in men with type 2 diabetes. (Figure 1).

3.4. F-tes Concentrations in Diabetic Retinopathy and Nephropathy

Based on the severity of diabetic retinopathy, serum F-tes

concentrations did not differ significantly for patients with NDR, SDR, and PDR (9.8 ± 4.1 vs. 9.7 ± 3.8 vs. 9.5 ± 3.1 pg/ml respectively). Similarly, based on the severity of diabetic nephropathy serum F-tes concentrations did not differ significantly for patients with normoalbuminuria, microalbuminuria, or macroalbuminuria (9.9 ± 4.3 vs. 10.2 ± 4.1 vs. 10.0 ± 3.6 pg/ml, respectively).

Table 1. Demographic and clinical characteristics of the patients

Variable	Type 2 diabetes patients (123 men)
Age (years) [mean $\pm$ SD]	60.0 $\pm$ 7.9
Age at onset (years) [mean $\pm$ SD]	49.0 $\pm$ 9.7
Duration of diabetes (years) [mean $\pm$ SD]	11.2 $\pm$ 7.1
BMI (kg/m ²) [mean $\pm$ SD]	29.11 $\pm$ 3.41
HbA _{1c} (%)[mean $\pm$ SD]	7.5 $\pm$ 1.6
Systolic blood pressure (mmHg) [mean $\pm$ SD]	146.70 $\pm$ 9.02
Diastolic blood pressure (mmHg) [mean $\pm$ SD]	91.76 $\pm$ 9.36
Total cholesterol (mg/dl) [mean $\pm$ SD]	194.84 $\pm$ 33.20
Triglyceride (mg/dl) [mean $\pm$ SD]	151.42 $\pm$ 15.84
HDL cholesterol (mg/dl) [mean $\pm$ SD]	38.38 $\pm$ 3.07
Retinopathy (NDR/SDR/PDR) [Number]	68/32/23
Nephropathy [Number] (normoalbuminuria/microalbuminuria/macroalb uminuria)	70/31/22
F-tes (pg/ml) [mean $\pm$ SD]	10.6 $\pm$ 3.8

BMI, Body Mass Index; Hb, Haemoglobin; HDL, High Density Lipoprotein; NDR, no diabetic retinopathy; SDR, simple diabetic retinopathy; PDR, proliferative diabetic retinopathy; IMT, intima-media thickness; F-tes ,serum free testosterone.

Table 2. Carotid duplex measurement of IMT of the patients

IMT in low F-tes [mean $\pm$ SD] mm	IMT in high F-tes [mean $\pm$ SD] mm	<i>p</i>
1.03 $\pm$ 0.26	0.88 $\pm$ 0.23	0.038

IMT, intima-media thickness

Table 3. Correlation coefficient between serum testosterone and other variables

Parameter	<i>r</i>	<i>p</i>
Age	-0.410	<0.0001
Age at onset	-0.277	<0.0001
Duration of diabetes	-0.171	0.0104
BMI	-0.765	0.0001
HbA _{1c}	-0.119	0.0409
Systolic blood pressure	-0.601	0.0001
Diastolic blood pressure	-0.457	0.001
Total cholesterol	-0.431	0.002
Triglyceride	-0.118	0.414
HDL cholesterol	0.114	0.430
IMT	-0.196	0.0093

BMI, Body Mass Index; HbA_{1c}, Haemoglobin A1c; HDL, High Density Lipoprotein; IMT, intima media thickness

Figure 1. Correlation between concentration of F-tes and carotid mean IMT

4. Discussion

In this study we tried to disclose the relationships between serum testosterone concentration and carotid atherosclerosis and its risk factors in men with type 2 diabetes. The relationship between serum testosterone concentration and carotid atherosclerosis was determined by using ultrasonographic measurement of carotid intima media thickness (IMT) in men with type 2 diabetes. Serum F-tes concentrations were negatively correlated with mean IMT. Data revealed that patients with low concentrations of F-tes had greater IMT than those with high concentrations of F-tes. This is found to be in agreement with results of other reports (1, 16, 17, 18, 19). This can be explained by the fact that testosterone is thought to modulate vascular smooth muscle cells (VSMC) through the up regulation of proliferation genes and through possible inhibitory interactions with intracellular signaling pathways induced by pro-inflammatory cytokines, decreasing inflammation-induced apoptosis and promoting proliferation (1, 20).

We revealed a negative correlation between F-tes concentrations and total cholesterol level. This is in agreement with Barud et al and Akinloye et al (21,22). Other reports stated that testosterone therapy could improve dyslipidemia (23,24). This could be attributed to the fact that cholesterol is the immediate biosynthetic precursor of steroids. Accordingly, low concentrations of testosterone might lead to increased concentrations of cholesterol by changing body fat distribution (25).

Our data showed a negative correlation between serum F-tes concentration and BMI. This was found to be consistent with other reports (22, 23, 24, 26, 27, 28), which confirmed an inverse association between testosterone levels and obesity, increased percentage body fat, insulin resistance and dyslipidemia and trunkal obesity. Moreover, other studies demonstrated that testosterone therapy in obese men reduces body mass index (BMI) and visceral fat mass (29,30).

We searched for the effect of testosterone on glycemic control in diabetic men patients. A relationship between serum concentrations of testosterone with HbA_{1c} level was established. Our result showed a negative correlation between F-tes and HbA_{1c}. This was in agreement with Fukui et al and Jones et al (24, 31) who demonstrated that a benefit on HbA_{1c} of testosterone therapy compared to placebo was observed after 9 months. This can be explained by influences of testosterone on the insulin signaling pathway (32). Testosterone was observed to elevate the expression levels and stimulate translocation of Glut4 in cultured skeletal muscle cells and to upregulate Glut4 by activating insulin receptor signaling pathways in neonatal rats (33). Furthermore, the presence of dyslipidaemia in the body further impairs insulin sensitivity, which is related to insulin resistance (34).

The presence of low free testosterone levels in our patients suggests that testosterone insufficiency may be a risk factor for type 2 diabetes. This is found to be comparable with other studies (35, 36). Men with diabetes have significantly lower plasma concentrations of F-tes than nondiabetic men. This is can be explained by the conversion of testosterone to estradiol by the actions of aromatase in adipose tissue. Therefore, a reduction of testosterone is inevitable with increased expression of aromatase, which is a result of an increased number of adipocytes in diabetic men. In addition, the normal negative feedback regulation of testosterone depends mainly on its aromatization to estradiol. Thus, a high level of aromatization results in the suppression of testosterone secretion. Falling testosterone promotes increasing adipocyte number and fat deposition which gradually leads to a further lowering effect on testosterone levels. In addition, the majority of the normal negative feedback of testosterone on the hypothalamo-pituitary axis occurs via its aromatization to oestradiol (32, 34). Thus, the increased risk for CVD in diabetic men could be partially mediated through low concentrations of testosterone.

Our results displayed a negative correlation between testosterone level and systolic and diastolic blood pressure. This is in agreement with Akinloye et al (22). Explanation of that is the steroid-induced vasodilation mediated via both membrane subpopulations of nuclear steroid receptors, such as the androgen receptor (AR), (36) as well as novel G protein- coupled receptors (38). Chignalia et al presented new evidence on genomic and nongenomic mechanisms of testosterone action on vascular smooth muscle cells in arterial hypertension through modulating associated cellular events (39).

5. Conclusion

In conclusion, serum free testosterone concentration was found to be low in type 2 diabetic men. It has a negative correlation with patients' age, age at onset of the disease, duration of diabetes, BMI and HbA_{1c}, total cholesterol concentration, systolic and diastolic blood pressure and

mean IMT. This may disclose the different mechanisms played by testosterone in the pathogenesis of cardiovascular risk in men with type 2 diabetes.

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