Original Article

Association of Low Serum Total Testosterone with Carotid Atherosclerosis in Male

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Abstract:
The present study was aimed to evaluate the association of serum total testosterone in 59 patients of carotid atherosclerotic male [<50% stenosis (n=26); >50% stenosis (n=33)]. Twenty-seven patients who had normal carotid doppler findings were taken as control. Serum total testosterone was measured by chemiluminescence micro particle immunoassay. Concentration of total testosterone differed significantly among groups (p<0.001) and it was significantly lower in both <50% and >50% stenosed group. Logistic regression analysis revealed that low total testosterone (total testosterone ≤8 nmol/L) was independently associated with development of carotid atherosclerosis (p<0.01, OR 5.03, 95% CI 1.22-20.64). In conclusion total testosterone is associated with carotid atherosclerosis in male.

Key words: Total testosterone, Carotid atherosclerosis

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Introduction:
Atherosclerosis underlies the pathogenesis of coronary, cerebral and peripheral vascular disease and causes more morbidity & mortality (Roughly half of all death)[1]. The South Asian countries like India, Pakistan, Bangladesh, Srilanka and Nepal contributes the highest proportion of the burden of cardiovascular disease (CVD) compared to any other region globally[2]. Over 80% of CVD takes place in this region[3]. According to health bulletin 2014, published by ministry of health and family welfare, Bangladesh, death caused by disease of circulatory system (33.2%) was highest among all cause of death[4].

Atherosclerosis is a chronic condition where the arteries become narrowed and hardened due to excessive buildup of plaque around the arterial wall, containing cholesterol, lipoid materials and macrophage[5]. Atherosclerotic events begin in childhood and remain clinically silent until they become large enough to impair tissue perfusion or until ulceration and disruption of the lesion, result in thrombotic occlusion or distal embolization of the vessel[6].

There are some well-known non modifiable and modifiable risk factors of atherosclerosis. But traditional risk factors fall short in identifying individuals at high risk for atherosclerosis[7]. So, there is continuous search for biomarkers which are easy to measure, standardize and independent from establish risk factors[8]. An atheromatous plaque consists of a grumous core of lipid (mainly cholesterol and cholesterol ester) covered by a white fibrous cap[1]. For many years physician only look for dyslipidemia in their patients with atherosclerosis. In a study of 27,939 healthy American, 77% of first cardiovascular events occurred in those with only moderately elevated low-density lipoprotein cholesterol (LDL-C) and 46% occurred among those with normal levels of LDL-C. Moreover, as many as 50% of first cardiovascular events occur in individuals with neither elevated cholesterol nor any other traditional risk factors[9].

Testosterone is a principal hormone that mainly bound two plasma protein sex hormone binding globulin and albumin[10]. Recently low testosterone has received increase attention not only from the standpoint of hypogonadism related symptoms but also increase cardiovascular mortality in men[11]. However lower testosterone has also been found in men with hypertension, stroke[12] insulin resistance and diabetes mellitus[13].

It is well known that vascular cell adhesion molecule 1 (VCAM-1) plays an important role in the development of atherosclerosis[14]. In vitro experiment revealed that testosterone restrained endothelial cells from excreting VCAM-1 by preventing nuclear factor kappa B from activation[15].

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Therefore, appropriate testosterone levels might inhibit the elevation of VCAM-1 expression and this way it might improve endothelial function[16]. Free testosterone and testosterone nonspecifically bound to albumin have been together called bioavailable testosterone (BT). BT reflects the physiological activity of testosterone but BT is difficult to measure in clinical setting and total testosterone replaces that of BT with exclusion of liver, renal and thyroid disorders[17]. In studies of middle-aged and older men, low total and free testosterone concentrations were associated with higher overall mortality and mortality from cardiovascular, cancer and respiratory causes[18-20]. However, other studies have reported negative or conflicting findings[21-23].

The potential role of low testosterone in the development of carotid atherosclerosis in men has been matter specific interest in recent years. That is why we designed a study was aimed to evaluate the association serum total testosterone with carotid atherosclerosis in men.

Materials & Methods:
The study was conducted from March 2015 to February 2016 after receiving Institutional Review Board approval from Bangabandhu Sheikh Mujib Medical University (BSMMU). By convenient and purposive sampling, a total of 86 male of age between 40-65 years attending in Radiology & Imaging department of BSMMU & National Institute of Neurosciences (NINS) for carotid doppler study, were enrolled in this study. The study subjects with liver disease, renal disease, thyroid disease, castration or taking any medication known to affect testosterone concentration (e.g. anti androgenic agents for prostate cancer) were excluded from this study.

After enrollment, they were grouped on the basis of doppler findings. Among them 27 were normal carotid doppler findings (group-I), 26 had stenosis upto 50% (group-II) and 33 had >50% stenosis (group-III). Informed written consents were taken and with all aseptic precaution, fasting blood samples were collected from each study subjects. Initial evaluation of the study subjects by history and clinical examination was performed and were recorded in the preformed data collection sheet. Demographic profile and pulse, BP, height, weight, BMI, WHR etc. were measured. Serum glucose (3.5-6 millimole/L) was measured using glucose oxidase method[24]. Serum creatinine (0.7-1.4 mg/dL) was measured by modified kinetic Jaffe assay[25].

The eGFR was calculated from serum creatinine for each subject. Serum alanine amino transferase (ALT), lipid profile, and total testosterone were measured by chemiluminescence micro particle immuno-assay[26]. Low total testosterone was defined as a serum total testosterone level below 8 nmol/L in accordance with the generally accepted standard[27].

The statistical analysis was carried out using the SPSS version 22. Quantitative data were expressed as mean and standard deviation (mean±SD). Differences among the groups were analyzed using ANOVA test, as well as the Chi-square test for categorical values. Differences between groups were assessed by mean of Mann Whitney U test or Bonferroni post hoc test as adequate. Multinomial regression analysis was performed to evaluate the relationships between carotid atherosclerosis and low total testosterone & also evaluate the odds ratio (OR), controlling for covariates. Spearman rank correlation coefficient was used to analyze correlation between total testosterone and carotid atherosclerosis. The p value <0.05 was considered significant.

Results:
This study was a cross sectional study. The study subjects were included who came for carotid doppler scanning. After getting the reports of the doppler study, the subjects were categorized into groups according to the reports. The study subjects who had normal sonographic findings were grouped into group-I (n=27), the study subjects who had up to 50% stenosis were grouped into group-II (n=26), and the study subjects who had >50% stenosis were grouped into group-III (n=33) (Table-I).

Table-I: Grouping of study subjects on the basis of ultrasonographic findings

Ultrasonographic findings	Number of Patients	%
Normal sonographic findings, Group-I	27	31.39
Stenosis up to 50%, Group=II	26	30.23
Stenosis > 50%, Group-III	33	38.37

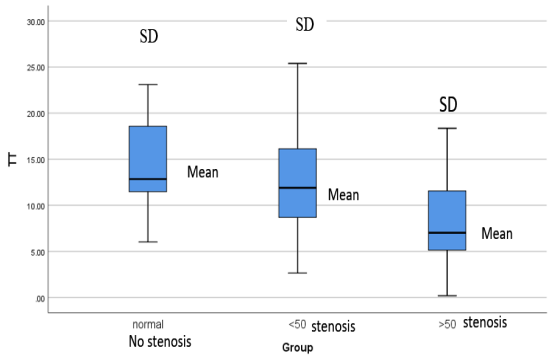

Figure-1: Mean distribution of free testosterone in different groups of study subjects

There were no significant differences among groups in term of overall baseline demographic, paraclinical, and clinical characteristics except smoking, which was statistically significant having p value 0.001 and significant was in group-III compared to group-I and II (Table II).

The concentration of serum total testosterone (nmol/L) differed significantly among groups (p=0.001) and it was significant in both <50% stenosed and >50% stenosed group (p=0.013 and p=0.001) (Table-III).

Figure-1 showed the mean distribution of total testosterone in different groups of study subjects. It showed the decrease trend of total testosterone with severity of atherosclerosis. Spearman's correlation test revealed that there was a significant negative correlation between total testosterone and atherosclerosis severity (y=-0.448 and p=<0.001) (Figure-2).

Multinomial logistic regression analysis of serum total testosterone, age, hypertension, DM, smoking, BMI, TC, HDL-C in relation to atherosclerosis severity, revealed that serum total testosterone (≤8 nmol/L) was independently associated with development of carotid atherosclerosis (p=0.025, OR 5.03, 95% CI 1.22-20.64) irrespective of the DM, HTN, smoking, TC, LDL-C, BMI and age of the study subjects (Table-IV).

Table-II: Sex distribution and episode of fever in the study population

Parameters	Group I n=27	Group II n=26	Group III n=33	p value
Age (years)	53.26±7.28	56.23±9.20	53.97±7.87	0.383
Smoking (n=35) (%)	29.6	19	66.6	0.001[a,b]
DM (n=26) (%)	18.5	30.7	39.3	0.125[a,b]
HTN (n=25) (%)	25.9	34.6	27.2	0.752[a,b]
BMI (kg/m^2)	23.51±2.94	24.64±1.55	24.86±3.65	0.117
WHR	0.98±0.05	0.96±0.04	0.99±0.04	0.132
SBP (mm of Hg)	117.88±17.50	127.12±19.01	122.41±19.64	0.173
DBP (mm of Hg)	78.26±6.29	79.81±7.41	74.85±10.42	0.078
FBS (mmol/L)	5.54±1.67	6.26±1.77	7.23±4.45	0.109
eGFR (ml/min/1.72m^2)	93.81±15,65	95.34±16.26	86.27±18.12	0.086
ALT (U/L)	31.48±14.45	31.85±15.62	37.64±16.70	0.391
Total cholesterol (mg/dL)	134.81±53.50	140.15±57.10	129.45±50.80	0.748
LDL-C (mg/dL)	77.77±37.44	84.9±43.19	77.38±41.04	0.744
HDL-C (mg/dL)	25.44±9.86	25.26±9.39	23.18±9.97	0.604

Continuous variables reported as mean ±SD and categorical variables as absolute or relative frequencies; ANOVA test was done to find out the level of significance; [a]Bonferroni post hoc test was done to find out exact level of significance; [b]Chi-square test was done to find out the level of significance.

Table-III: Comparison of the serum total testosterone among the groups

Groups	TT (nmol/L) Mean±SD	p value
Group I	14.30±5.07	0.001
Group II	12.04±5.73	
Group III	8.12±4.62	

ANOVA test was done to find out the level of significance; To find out the exact level of significance Bonferroni post hoc test was done which showed that the statistically significant level is between group I & group III (p=0.001) and between group II & group III (p= 0.013).

Table-IV: Logistic regression analysis of Age, TT, HTN, DM, Smoking, BMI, TC, HDL-C with carotid atherosclerosis

Variable of interest	OR	95% CI of odds	p value
Age (≥55 years)	2.71	0.84 - 5.05	0.093
TT (≤ 8 nmol/L)	5.03	1.22 – 20.64	0.025
HTN (Yes)	1.31	0.38 - 4.53	0.662
DM (Yes)	3.21	0.87 - 11.78	0.078
Smoking (Yes)	1.52	0.46 - 4.97	0.489
BMI (≤25 kg/m^2)	0.34	0.11 - 1.45	0.060
TC (>200 mg/dL)	5.36	1.23 - 23.29	0.025
HDL-C (≤30 mg/dL)	1.56	0.48 - 5.05	0.458

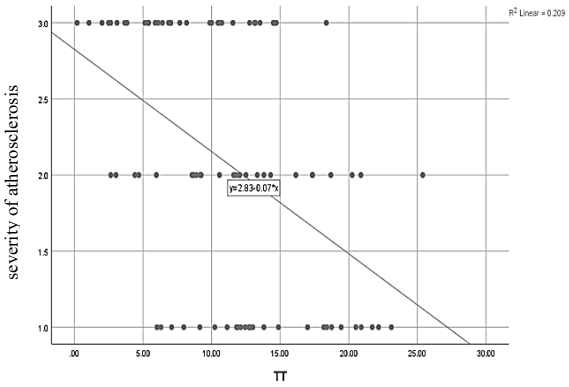

Figure-2: Correlation between total testosterone (TT) and severity of atherosclerosis. (Correlation coefficient r = -0.448)

Discussion:

This study was aimed to evaluate the association of serum total testosterone with carotid atherosclerosis in male. The study subjects were a cross section of population who came for carotid doppler and blood samples were collected for the measurement of the markers of interest. We enrolled 86 male patients and among them 27 were normal (group-I), 26 had stenosis up to 50% (group-II) and 33 had >50% stenosis (group-III) diagnosed by carotid doppler.

Mean age in the three groups were not statistically significant which reflects homogeneity of the groups in term of age. Our results in consistent with study done by Makinen et al.[28].

In our study we compared baseline demographic, paraclinical and clinical characteristics of the study subjects grouped into three groups in term of BMI (kg/m^2), WHR, BP, FBS (mmol/L), eGFR ($ml/min/1.72m^2$) and lipid profile. None of them were statistically significant. The mean LDL-C value of the three groups were not high (<100 mg/dL) which supports observation of Libby et al.[29], that only dyslipidaemia is not enough to find out the risk of atherosclerosis in an individual.

We investigate the distribution of study subjects in term of number of smokers, diabetes mellitus and hypertensive patients in three groups. Though numbers of DM and HTN individuals were not significant in three groups but smoker were significantly differed with each other. Soisson et al.[30] and Tsujimura et al.[31] did not find the association of smoker with severity of atherosclerosis but Svartberg et al.[32] found the association of smoking severity of atherosclerosis.

The total testosterone levels were lower both in individual with <50% carotid atherosclerosis and >50% stenosis than normal carotid doppler findings and statistically significant difference was found in between no stenosis and <50% stenosis & between <50% stenosed and >50% stenosed group. Other researchers found a significant inverse correlation with total testosterone and carotid atherosclerosis[33,34,35] and is consistent with the concept that total testosterone reflects total availability of testosterone to the target tissue[36]. Contrary to our results, Vikan et al.[37] found that there is no significant association between endogenous testosterone progression of carotid atherosclerosis.

We also did the Spearman's correlation test which showed significant negative correlation between serum total testosterone and severity of carotid atherosclerosis but findings were not compatible with that of the study of Vikan et al.[37].

Finally, we did multinomial logistic regression analysis which showed that low levels of serum total testosterone (≤8 nmol/L) were independently associated with the development of carotid atherosclerosis in male. This result suggested that total testosterone deficiency is the sole contributing factor in developing carotid atherosclerosis irrespective to age, BMI, DM, HTN, smoker, TC and HDL-C.

Our study result was not coherent with the findings of Pergola et al.[38] who conducted a large cross-sectional study of carotid atherosclerosis subjects. Another cross-sectional study of negatively associated testosterone with carotid atherosclerosis subjects conducted by Glisic et al.[39] also supported the findings of Pergola et al.[38] and this conflicted with our study result. These dissimilarities of these two study results with our study findings indicate that a multi-institutional large sample case-control study is needed to be conducted.

Conclusion:

Serum low total testosterone is independently associated with carotid atherosclerosis in male. However, the relationship between low total testosterone and carotid atherosclerosis still remains inconclusive.

References:

1. Mitchell RN. Blood vessel. In: Kumar V, Abbas AK, Aster JC, Eds. Robins and Cortan pathologic basis of disease, 9th ed., USA: Elsevier Saunders Philadelphia; 2015. p 483-521.
2. Wasay M, Khatri IA, Kaul S. Stroke in South Asian countries. Nat Rev Neurol. 2014; 10 (3): 135-43.
3. World Health Organization. World health statistics 2012. p 132-5. Available at: https://www.who.int/gho/publications/world_health_statistics/EN_WHS2012_Full.pdf [Accessed on January10, 2019]

4. Ministry of health and family welfare Bangladesh. Health bulletin 2014, 2nd ed. p 30-59. Available at https://dghs.gov.bd/images /docs/Publicaations/HB_2014_2nd_Edition_06 0115.pdf [Accessed on January 11, 2019]
5. Lusis A. Atherosclerosis. Nature. 2000; 407 (6801): 233-41.
6. Langhorne P. Stoke disease. In: Walker BR, Colledge NR, Ralston SH, Penman ID, Eds. Davidsons principles and practice of medicine, 22th ed., China: Elsevier; 2014. p 1232-47.
7. Yeh ETH. High sensitivity C - reactive protein as a risk assessment tool for cardiovascular disease. Clin Cardiol. 2005; 28 (9): 408-12.
8. Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, et al. Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation. 2003; 107 (3): 499-511.
9. Ridker M, Rifai N, Rose L, Buring E, Cook NR. Comparison of C-reactive protein and low-density lipoprotein cholesterol level in the prediction of first cardiovascular events. N Engl J Med. 2002; 347 (20): 1557-65.
10. Swerdloff RS, Wang C. The testes and male sexual hormone. In: Ausiello G, Ed. Cecil Medicine, 23rd ed., USA: Elsevier Philadelphia; 2008. p 2210-45.
11. Haring R, Volzke H, Steveling A, Krebs A, Felix SB, Schofl C, et al. Low serum testosterone levels are associated with increased risk of mortality in a population-based cohort of men aged 20-79. Eur Heart J. 2010; 31 (12): 1494-1501.
12. Svartberg J, Muhlen DV, Schirmer H, Barrrett-Connor E, Sundfjord J, Jorde R. Association of endogenous testosterone with blood pressure and left ventricular mass in men: The Tromso study. Eur J Endocrinol. 2004; 150 (1): 65-71.
13. Mattack N, Devi R, Kutum T, Patgiri D. The evaluation of serum levels of testosterone in type 2 diabetic men and its relation with lipid profile. J Clin Diagn Res. 2015; 9 (1): 4-7.
14. Malkin CJ, Pugh PJ, Jones RD, Jones TH, Channer KS. Testosterone as a protective factor against atherosclerosis - immunomodulation and influence upon plaque development and stability. J Endocrinol. 2003; 178 (3): 373-80.
15. Hatakeyama H, Nishizawa M, Nakagawa A, Nakano S, Kigoshi T, Uchida K. Testosterone inhibit tumor necrosis factor Alfa-induced vascular cell adhesion molecule-1 expression in human aortic endothelial cells. FEBS Lett. 2002; 530 (1-3): 129-32.
16. Fu L, Gao QP, Shen JX. Relationship between testosterone and indexes indicating endothelial function in male coronary heart disease patients. Asian J Androl. 2008; 10 (2): 214-8.
17. Morales A, Lunenfeld B. Investigation, treatment and monitoring of late-onset hypogonadism in males. Official recommendations of ISSAM. International Society for the Study of the Aging Male. Aging Male. 2002; 5 (2): 74-86.
18. Shores MM, Matsumoto AM Sloan KL, Kivlahan DR. Low serum testosterone and mortality in male veterans. Arch Intern Med. 2006; 166 (15): 1660-5.
19. Khaw KT, Dowsett M, Folkerd E, Bingham S, Wareham N, Luben R, et al. Endogenous testosterone and mortality due to all causes, cardiovascular disease and cancer in men. Circulation 2007; 116 (23): 2694-701.
20. Laughlin GA, Barrett-Connor E, Bergstrom J. Low serum testosterone and mortality in older men. J Clin Endocrinol Metab. 2008; 93 (1): 68-75.
21. Morley JE, Kaiser FE, Perry 3rd HM, Patrick P, Morley PM, Stauber PM, et al. Longitudinal changes in testosterone, luteinizing hormone and follicle stimulating hormone in healthy older men. Metabolism. 1997; 46 (4): 410-3.
22. Smith GD, Ben-Shlomo Y, Beswick A, Yarnell J, Lightman S, Elwood P. Cortisol, testosterone and coronary heart disease: protective evidence from the caerphilly study. Circulation. 2005; 112 (3): 332-40.
23. Araujo AB, Kupelian V, Page ST, Handelsman DJ, Bremner WJ, Mckinlay JB. Sex steroid and all cause and cause-specific mortality in men. Arch Intern Med. 2007; 167 (12): 1252-60.
24. Barham D, Trinder P. An improved colour reagent for the determination of blood glucose by glucose oxidase system. Analyst. 1972; 97 (151): 142-5.
25. Murray RL. Ceratinine. Clin Chem. 1984; 418: 1261-66.
26. Architect Abbott Diagnostics. Estimation of ALT, Lipid profile, Testosterone. In: Operation manual for architect Ci 4100, 2013; Abbott laboratories, USA.
27. Isbell TS, Jungheim E, Gronowski AM. Reproductive endocrinology and related disorders. In: Burtis CA, Ashwood ER, Brruns DE, Eds. Clinical chemistry and molecular diagnostics, 5th ed. USA: Elsevier; 2012. p 1945-90.
28. Makinen J, Jarvisalo MJ, Pollanen P, A Perheentupa, Irjala K, Koskenvuo M, et al. Increased carotid atherosclerosis in andropausal middle aged men. J Am Coll Cardiol. 2005; 45 (10): 1603-8.
29. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation. 2002: 105 (9): 1135-43.

30. Soisson V, Brailly-Tabard S, Empana JP, Feart C, Ryan J, Bertrand M, et al. Low plasma testosterone and elevated carotid intima-media thickness: Importance of low-grade inflammation in elderly men. Atherosclerosis. 2012; 223 (1): 244-9.
31. Tsujimura A, Matsumiya K, Matsuoka Y, Takahashi T, Koga M, Iwasa A, et al. Bioavailable testosterone with age and erectile dysfunction. J Urol. 2003; 170 (6 Pt 1): 2345-7.
32. Svartberg J, von-Muhlen D, Mathiesen E, Joakimsen O, Bonaa KH, Stensland-Bugge E. Low testosterone levels are associated with carotid atherosclerosis in men. J Intern Med. 2006; 259 (6): 576-82.
33. Chan YX, Knuiman MW, Hung J, Divitini ML, Handelsman DJ, Beilby JP, et al. Testosterone, dihydrotestosterone and estradiol are differentially associated with carotid intima-media thickness and the presence of carotid plaque in men with or without coronary artery disease. Endocr J. 2015; 62 (9): 777-86.
34. Dörr M, Wallaschofski H, Friedrich N. Association of low total testosterone levels and prevalent carotid plaques: result of the study of health in Pomerania. Eur J Epidemiol. 2009; 24 (7): 389-91.
35. Kwon H, Lee DG, Kang HC, Lee JH. The relationship between testosterone, metabolic syndrome and carotid intima-media thickness in ageing men. Aging male. 2014; 17 (4): 211-5.
36. Fukui M, Kitagawa Y, Nakamura N, Kadono M, Moganni S, Hirata C, et al. Association between serum testosterone and carotid atherosclerosis in men with type 2 diabetes. Diabetes Care. 2003; 26 (6): 1869-73.
37. Vikan T, Jhonsen SH, Schirmer H, Njolstad I, Svartberg J. Endogenous testosterone and the prospective association with carotid atherosclerosis in men: The Tromso study. Euro J Epidemiol. 2009; 24 (6): 289-95.
38. Pergola G, Pannacciulli N, Ciccone M, Tartagni m, Rizzan P, Giorgino R. Free testosterone plasma levels are negatively associated with the intima-media thickness of the common carotid artery in overweight and obese glucose-tolerant young adult men. Int J Obes Relat Metab Disord. 2003; 27 (7): 803-7.
39. Glisic M, Mujaj B, Rueda-Ochoa OL, Asllanaj E, Laven JS, Kavousi M, et al. Association of endogenous estradiol and testosterone levels with plaque composition and risk stroke in subjects with carotid atherosclerosis. Circ Res. 2018; 122 (1): 97-105.

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