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The association between low serum total testosterone and atherosclerotic cardiovascular disease

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Cardiovascular disease is the leading cause of mortality and morbidity globally. Recent studies have demonstrated that low testosterone in men tends to be a potent risk factor for cardiovascular diseases. However, the association between the testosterone and severity of the cardiovascular disease remains inconclusive. This study has evaluated testosterone as an independent marker for atherosclerotic cardiovascular disease in North Eastern Indian Population. This study was taken up to assess the correlation between serum testosterone and atherosclerotic cardiovascular disease and to find the association between serum testosterone levels and severity of coronary artery disease by Gensini score. This Cross-sectional study was conducted in NEIGRIHMS, Shillong involving 160 participants. Serum total testosterone levels were estimated along with other biochemical parameters. Gensini score and ejection fraction were calculated by cardiologist based on angiographic finding and echocardiograph respectively. Correlations between serum testosterone levels and Gensini scores were assessed using Pearson's correlation and a multivariate logistic regression analysis was done to find the association of testosterone and cardiovascular disease. The mean serum total testosterone was significantly lower in the case group (3.49 ± 1.48) *vs* Controls (5.87 ± 1.25) ($P< 0.001$). A multiple logistic regression analysis demonstrated that low testosterone is an independent risk factor for CAD ($P< 0.001$). ROC analysis yielded an AUC of 0.886 with a P value of <0.001 with sensitivity 70% and specificity of 95.1% of serum total testosterone. The study demonstrates an inverse association between serum total testosterone and atherosclerotic cardiovascular disease, measurable through the Gensini score.

Keywords: Coronary artery disease, Endothelial dysfunction, GENSINI score, Testosterone

Atherosclerotic Cardiovascular disease is the leading cause of global mortality and morbidity. In that coronary artery disease serves as the primary contributor[1]. Hypogonadism is not generally considered to be a risk factor for coronary artery disease. Studies have shown that as age increases testosterone level decreases in men[2-5]. One of the traditional and strong risk factors for atherosclerotic cardiovascular disease is male sex. In the recent past the recognition of the nontraditional coronary artery disease risk factors have been linked with inflammatory and pro coagulant states[6]. With this context studies have shown that hypogonadal males exhibit an increased prevalence of coronary artery disease. These two phenomena have triggered researches to find the association between the serum levels of testosterone and coronary artery disease.

Testosterone normally exist in two different forms in serum. Nearly 68% of the testosterone is tightly bound to the sex hormone binding globulin and remains biologically inactive. The remaining testosterone is termed as bioavailable testosterone. Around 30% of the bioavailable testosterone is loosely bound to the albumin and remaining 1% to 3% of testosterone circulate freely in the serum. This free form of testosterone is termed as free testosterone. The sum of all the testosterone subfractions is called as total testosterone[7,8].

Researchers have shown that testosterone has vaso-protective effects on both coronary blood flow and systemic blood circulation[9]. In spite of evidences showing a link between low testosterone levels and coronary artery disease, studies have shown contradictory results on the effect of testosterone on coronary artery disease[10]. This study is taken up to find the association between serum testosterone and atherosclerotic cardiovascular disease and determine the severity of coronary artery disease based on testosterone levels.

Materials and Methods

This was a cross-sectional study conducted in the Department of Biochemistry in collaboration with

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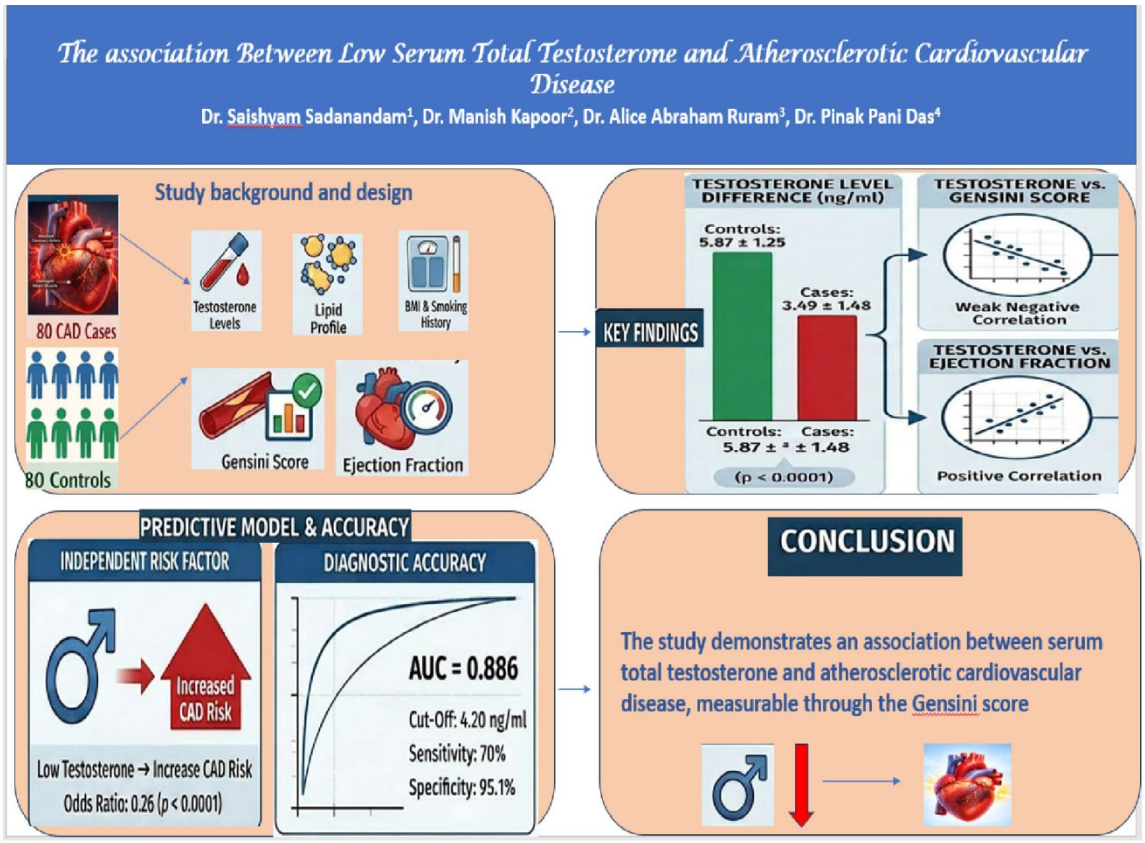

Graphical abstract

Department of Cardiology, NEIGRIHMS, Shillong from 2022 to 2024. A total of 160 male participants aged between 30 to 60 years were involved. Participants were divided into two groups, consisting of 80 male patients who are being evaluated for atherosclerotic cardiovascular disease for various spectrum of presentation and 80 males who had no evidence of CVD, came for master health check-up. Patients who had history of malignancy, recent infection, hypogonadism, liver and renal failure patients were excluded from the study. Serum total testosterone levels were measured by Roche diagnostics Cobas e601 based on the principle of electrochemiluminescence immunoassay. Gensini score was calculated by a cardiologist in case group by grading coronary stenosis as 1 (≤25%), 2 (26–50%), 4 (51–75%), 8 (76–90%), 16 (91–99%), and 32 (total occlusion), and multiplied by a factor based on lesion location in the coronary arterial tree. The final score was derived by adding the scores of all coronary lesions. Study was started after obtaining ethical clearance from institutional ethical committee and informed consent was obtained from the participants. Socio-demographic and clinical characteristics were recorded for each patient. A single overnight fasting venous blood of about 5 mL was collected in sterile plain vial from all the participants. Blood sample was centrifuged at 3000 rpm for 10 min to obtain serum which was used for the estimation of serum testosterone.

Continuous variables were expressed as mean value and standard deviation (SD). Categorical variables are presented as absolute values (percentages). The study population was divided into two groups. Comparisons between the groups pertaining to categorical data was done by chi-square test or *t*-test, whenever appropriate. Comparisons pertaining to quantitative data was done by *t*-tests. Correlations between serum testosterone levels and Gensini scores and ejection fraction was assessed using Pearson's correlation test. Similarly, correlation between serum testosterone and traditional risk factors for CAD was also assessed. A multivariate logistic regression analysis was done to assess whether serum testosterone is independently associated with CAD after adjusting for age, BMI, smoking history, hypertension, diabetes mellitus, dyslipidemia. Statistical significance is defined as a p

value less than 0.5. Data entry and analysis was done using SSPS version 22.

Results and Discussion

In the present study, the mean age of participants in the case group was (51.68± 9.24) when compared to the control group (51.52 ± 9.46) with a p- value of 0.914 suggesting successful matching with respect to age.

The mean total testosterone levels in case group (3.49 ± 1.48) were lower than the control group (5.87 ± 1.25) (Fig. 1, and Table 1). A highly statistically significant difference was seen in serum total testosterone levels between the case group and control group with p value of <0.001. This finding clearly indicates the presence of a strong association between lower endogenous testosterone levels and incidence of atherosclerotic cardiovascular disease. This finding is consistent with previous studies linking low levels of testosterone with cardiovascular disease. In

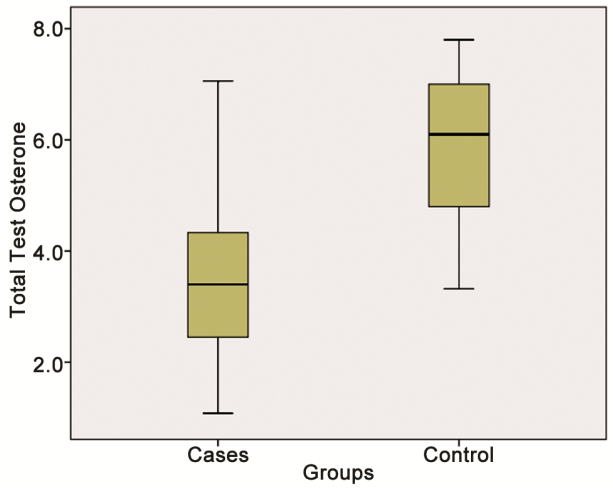

Fig. 1 — Box and Whisker Plot of Serum Total Testosterone in cases and control

corroboration with our findings, a study conducted by Gururani K *et al*, revealed that the levels of total, free and bioavailable testosterone were lower in the coronary artery disease group (363 ± 147.174 ng/dL) compared to the control group (532.9 ± 150.553 ng/dL) which was statistically significant (p value <0.01)[11]. Testosterone may have a protective effect on cardiovascular system (CVS) via its effect on vascular reactivity, immune modulation, arterial wall stiffness and the endothelium. Testosterone has been inversely correlated with vascular cell adhesion molecule-1 (VCAM-1), which is produced by endothelial cells and up-regulated when endothelial cells undergo inflammatory and malignant stimulation leading to atherosclerotic lesions formation[12]. It has also been reported to induce vasodilation by reducing calcium influx into vascular smooth muscle by acting as a selective and potent inhibitor of L-type calcium channels at physiologic levels and as an inhibitor of T-type calcium channels at supraphysiologic levels. Testosterone is negatively correlated with fibrinogen, which increases CVD risk through its effect on atherogenesis, thrombogenesis, and ischemia by increasing plasma and blood viscosity[13].

The case group shows a significantly higher levels of triglyceride, VLDL and BMI when compared to the control group (Table 1). The case group also has a significant higher prevalence of smoking (p= 0.002) (Table 2).

Gensini scoring system was used to assess the severity of coronary artery disease. Pearson correlation was used to find the possible association between the testosterone and the disease severity. Our study revealed a negative correlation between testosterone and Gensini score but not statistically significant (r = -0.91, p value 0.4179) (Table 3). This was found to be in agreement with the study

Table 1 — Baseline Characteristics of Study Participants

Variable	Case (Mean±SD)	Control (Mean±SD)	p-value
Age (years)	51.68 ± 9.24	51.52 ± 9.46	0.914
Total Testosterone (ng/mL) (Reference range 3-10 ng/mL)	3.49 ± 1.48	5.87 ± 1.25	<0.001
Triglyceride (mg/dL) (Reference range < 150 mg/dL)	132 ± 61.47	101.35 ± 31.54	0.001
VLDL (mg/dL) (Reference range 5- 40 mg/dL)	26.51 ± 12.24	20.32 ± 6.21	0.001
Total Cholesterol (mg/dL) (Reference range <200 mg/dL)	143.22 ± 28.99	138.56 ± 33.73	0.34
HDL (mg/dL) (Reference range 40- 60 mg/dL)	40.62 ± 7.90	42.32 ± 10.79	0.25
LDL (mg/dL) (Reference range <100 mg/dL)	76.09 ± 21.26	78.0 ± 30.43	0.64
BMI	24.70 ± 1.85	23.68 ± 1.14	<0.001

Baseline characteristics of the 160 participants are given in the table 1. P-values calculated using the independent samples t-test for continuous variables

Table 2 — Comparison of smoking history between cases and control

	Case (Mean±SD)	Control (Mean±SD)	p-value
Smoking (Yes)	35 (43.2%)	16 (19.5%)	0.002

P-value calculated using the Chi-squared test

Table 3 — Correlation of testosterone and disease severity (Case Group Only)

Variable	r-value (Pearson)	p-value
GENSINI Score (Severity)	-0.091	0.4179
Ejection Fraction (Function)	0.206	0.0628

This analysis focused on the relationship between testosterone and measures of CAD within the case group

Table 4 — Multivariate logistic regression analysis for testosterone as dependent variable

Predictor	Coefficient (log-odds)	Odds Ratio (OR)	P-value
Total Testosterone	-1.350	0.26	< 0.001
BMI	0.768	2.16	0.003
Smoking (Yes)	1.606	4.98	0.085
Triglyceride	0.015	1.02	0.583
Age	-0.016	0.98	0.5632

Table 5 — Area under the curve for total testosterone

Area	Std. Error[a]	Asymptotic Sig.	Asymptotic 95% confidence interval	
			Lower Bound	Upper Bound
0.886	0.26	<0.001	0.836	0.937

done by Bashyal R *et al*, in their study found that serum total testosterone was negatively correlated with the Gensini score but not statistically significant[14,15].

A moderate positive correlation was found between serum total testosterone and left ventricular ejection fraction. This suggests that patients with higher testosterone levels tend to have a slightly better heart function. This finding is similar to the study done by Dobrzycki S *et al*, in which they concluded that low levels of free-testosterone was characteristic for patients with low ejection fraction[16,17].

After controlling various traditional coronary artery risk factors such as BMI, smoking, age, triglyceride, a multiple logistic regression analysis demonstrated that low testosterone is an independent risk factor for CAD (P< 0.001) (Table 4). This finding is found to be similar to the study done by Gururani k *et al*[11]. To find the

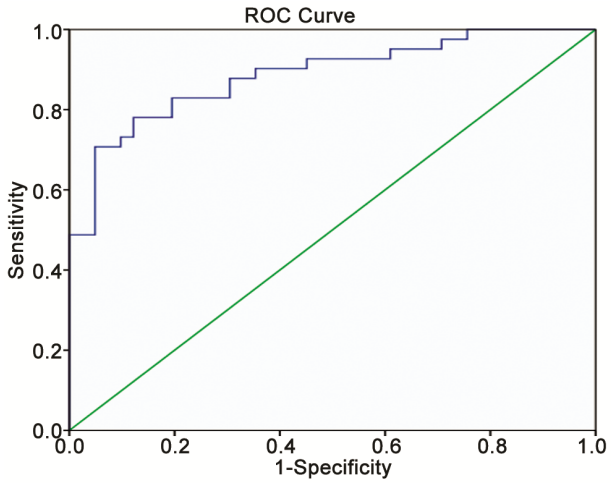

Fig. 2 — ROC curve analysis for serum total testosterone

diagnostic accuracy of testosterone receiver operating characteristic curve analysis was done. The area under the curve is found to be 0.886 with a P value of <0.001. At the optimal cut-off value of 4.20 the sensitivity and specificity of serum total testosterone was found to be 70% and 95.1% respectively (Fig. 2, and Table 5).

Conclusion

Our study demonstrates that low levels of serum total testosterone serve as an independent risk predictor for atherosclerotic cardiovascular disease in men. A negative correlation was found between the Gensini score and the serum total testosterone and a positive correlation was demonstrated between serum total testosterone and the left ventricular ejection fraction. Serum total testosterone may be used as a tool to find the risk of atherosclerotic cardiovascular disease in men.

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Conflict of interest

All authors declare no conflict of interest.

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