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Serum Testosterone Levels and Coronary Artery Disease

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Abstract: ***Introduction: Coronary Artery Disease (CAD) is the leading cause of death worldwide. Testosterone has protective effect on cardiovascular system (CVS). Men are more prone to develop CAD than women and the mechanism of this different susceptibility is not well understood. Objectives: To estimate the concentration of serum testosterone levels in male subjects with and without CAD and to determine the relationship between serum Testosterone levels and angiographic severity of CAD. Methodology: A cross - sectional study, consisting of 110 subjects out of which 55 subjects diagnosed with CAD and 55 healthy subjects, was carried out in the department of Biochemistry in collaboration with the department of Cardiology of RIMS, Imphal from January 2021 to October 2022. Serum free and total testosterone levels were measured and the severity of CAD was assessed using angiographic Gensini score. Result: The mean ± SD of serum total and free testosterone levels were significantly lower in case group (2.05 ± 1.18 ng/ml, 3.79 ± 2.71 pg/ml) when compared to control group (4.97 ± 0.65 ng/ml, 16.22 ± 4.60 pg/ml). Cases with Gensini score >30 had significantly lower total testosterone levels and free testosterone levels than cases whose Gensini score were ≤30. A multiple linear regression analysis showed that low testosterone was an independent risk factor the severity of CAD (β= - .238, p=.014). Conclusion: Men with coronary artery disease have significantly lower testosterone than normal controls. This study also suggests low serum testosterone as an independent risk factor for CAD.***

Keywords: Testosterone, Coronary Artery Disease, Gensini Score

1. Introduction

Cardiovascular disease (CVD) is one of the leading causes of morbidity and mortality in the world accounting for approximately 21% deaths in 2010 with 11% of deaths due to ischemic heart disease.[1] Coronary artery disease (synonym: Ischemic Heart Disease and Coronary Heart Disease) typically occurs when there is an imbalance between myocardial oxygen supply and demand. The most common cause is atherosclerotic disease of an epicardial coronary artery (or arteries).[2] The strongest independent risk factors for CAD are that of increased age and the male sex. A consistent male: female ratio of approximately 2: 1 is observed despite a wide variance in CAD mortality between countries. Serum testosterone level is also shown to be decreased with advancing age in men. Such data have led to the assumptions that male hormones, and particularly testosterone, may be involved in the pathogenesis of CAD.[3]

Testosterone (Ts) is a 19 carbon male sex steroid hormone with a hydroxyl group in the 17th position synthesized primarily by the Leydig cells of testes (95%) and, to a lesser extent (≈5%), via peripheral conversion from the precursors: dehydroepiandrosterone (DHEA) and androstenedione. Testosterone (Ts) may have a protective effect on cardiovascular system (CVS) via its effect on vascular reactivity, immune modulation, arterial wall stiffness and the endothelium.[4]

Knowledge and understanding of serum Ts level is gaining importance as men with CAD are at a higher risk of cardiovascular mortality and represent a patient population prone to testosterone deficiency. There are limited number of studies regarding serum testosterone level and CAD in this region. It is also hard to draw a definite conclusion in the causal relationship due to inconsistent findings from various studies. So this study has been undertaken to determine whether serum testosterone profile in a group of male subjects with coronary artery disease has any significant difference or correlation when compared with a similar group of male subjects with no coronary artery disease.

2. Materials and Methods

This was a cross - sectional study carried out in the Department of Biochemistry in collaboration with the Department of Cardiology, Regional Institute of Medical Sciences, Imphal from January 2021 to October 2022. Fifty - five male patients aged 40 years and above with new onset or recent onset clinician diagnosed Coronary Artery Disease and fifty - five healthy male subjects above 40 years without Coronary Artery Disease were enrolled.

Exclusion criteria were as follows: previous revascularisation procedure, Malignancy, Chronic inflammatory disease like Crohns Disease, recent or current infection like typhoid fever, under sex hormone replacement therapy, chronic kidney disease and chronic liver disease.

Written informed consent was obtained from all patients before sample collection. The study was approved by the Research Ethics Board, Institutional Ethics Committee (IEC), Regional Institute of Medical Sciences (RIMS), Imphal (Ref no. – A/206/REB - Comm (SP) /RIMS/2015/676/17/2020).

Laboratory measurements

Five ml of venous blood was taken immediately prior to coronary angiography between 8: 00 am and 9: 00 am after 12 hours fast and allowed to clot at room temperature. The clot was retracted, and serum separated by centrifugation at 2000 rpm for 10 minutes. The serum was stored at - 20^0 C till analysis for serum testosterone levels. The total and free testosterone was measured by enzyme immunoassay for quantitative determination (CALBIOTECH ELISA kit, India. Catalogue number: TE373S and CALBIOTECH ELISA kit, India. Catalogue number: FT178S). The reference range for testosterone were as follows: total testosterone - 3 - 9.5 ng/ml and free testosterone - 5 - 30 pg/ml.

Evaluation

Detailed socio - demographic and clinical characteristics were recorded for each patients including age, hypertension, diabetes, smoking status and drinking status. Smoking habits were categorised as never smoking and smoking (including formerly smoking or currently smoking). Weight and height were recorded and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters.

Gensini Coronary Score

The severity of coronary stenosis in patients was estimated by the Gensini coronary score.[51] The Gensini score is based on the number of stenotic coronary artery segments, including the degree of luminal narrowing and the localisation of the stenosis.[52] The Gensini system scores the narrowing of the coronary artery lumen as follows: 1%–25% narrowing=1; 26%–50% narrowing=2; 51%–75% narrowing=4; 76%–90% narrowing=8; 91%–99% narrowing=16; and total occlusion=32. The score is then multiplied by a factor that incorporates the importance of the lesion position in the coronary arterial tree as follows: ×5 for the left main coronary artery; ×2.5 for the proximal left anterior descending or left circumflex coronary artery; ×1.5 for the mid - segment of the left anterior descending; ×1 for the distal left anterior descending, right coronary artery or mid - distal left circumflex; and ×0.5 for any other arteries.[5]

3. Statistical Analysis

The collected data were analysed using IBM: SPSS version 21 for Windows (IBM Corp, AMOK, NY, USA). Results were reported as frequency along with percentages for the categorical variables and mean ± SD (standard deviation) for quantitative variables. Independent samples t - test, Chi - square test were used to compare means and Pearson's correlation and Spearman's rank correlation was used to find out the correlation between the variables. A multivariate regression analysis was done to assess whether serum testosterone was independently associated with CAD after adjusting for age, BMI, hypertension, diabetes, smoking history and alcohol consumption. A two - sided p - value<0.05 were considered as statistically significant for all tests.

4. Results

Table 1: Comparison of baseline, clinical and biochemical characteristics between cases and controls (N=110)

Variables	Cases (N=55)	Controls (N=55)	p - value
Age (years)	63.40±11.92	60.00±8.07	0.08
BMI (kg m^{-2})	29.23±3.43	20.34±1.72	0.000**
Hypertension [n (%)]	39 (70.9%)	32 (58.2)	0.232
Smoking [n (%)]	40 (70.7%)	32 (58.2%)	0.160
Alcohol consumption [n (%)]	30 (54.5%)	22 ((40%)	0.181
Diabetes [n (%)]	36 (65.4%)	21 ((38.2%)	0.007**
Total testosterone (ng/ml)	2.05 ± 1.18	4.97 ±.65	0.000**
Free testosterone (pg/ml)	3.79 ± 2.71	16.22 ± 4.60	0.000**

Baseline, clinical and biochemical characteristics:

Table - 1 shows the baseline, clinical and biochemical characteristics of the study populations. CAD group had greater mean age than controls, however it was not statistically significant as seen from table - 1 (63.40±11.92 years vs 60.00±8.07; p=0.08). Diabetes mellitus and BMI differed significantly among the two study groups (p<0.05). However, there were no statistically significant difference between the two groups for hypertension, smoking and alchohol consumption (p >0.05).

The mean values of serum total and free testosterone were significantly lower in patients with CAD when compared to controls (p<0.05).

Table 2: Comparison of serum Total Testosterone and free Testosterone levels within CAD group stratified by Gensini score (N=55)

Gensini score	≤30 (N =30)	≥30 (N=25)	p - value
Total testosterone (ng/ml)	2.51±1.11	1.50±1.04	0.001
Free testosterone (pg/ml)	5.73±1.95	1.47±1.31	0.000

Serum testosterone levels within CAD group stratified by Gensini score

Table 2 shows that Total and free testosterone levels among the cases were divided according to Gensini score taking the median value 30 as the cut - off point. The cases with Gensini score >30 had significantly lower total testosterone levels than cases whose Gensini score were ≤30.

Table 3: Correlation between serum total testosterone level and other variables of interest among cases (N=55).

Variables	Total Testosterone Pearson correlation (r)	p - value
Age	- 0.397	0.003**
BMI	- .324	0.016**
Gensini score	- .509	0.000**

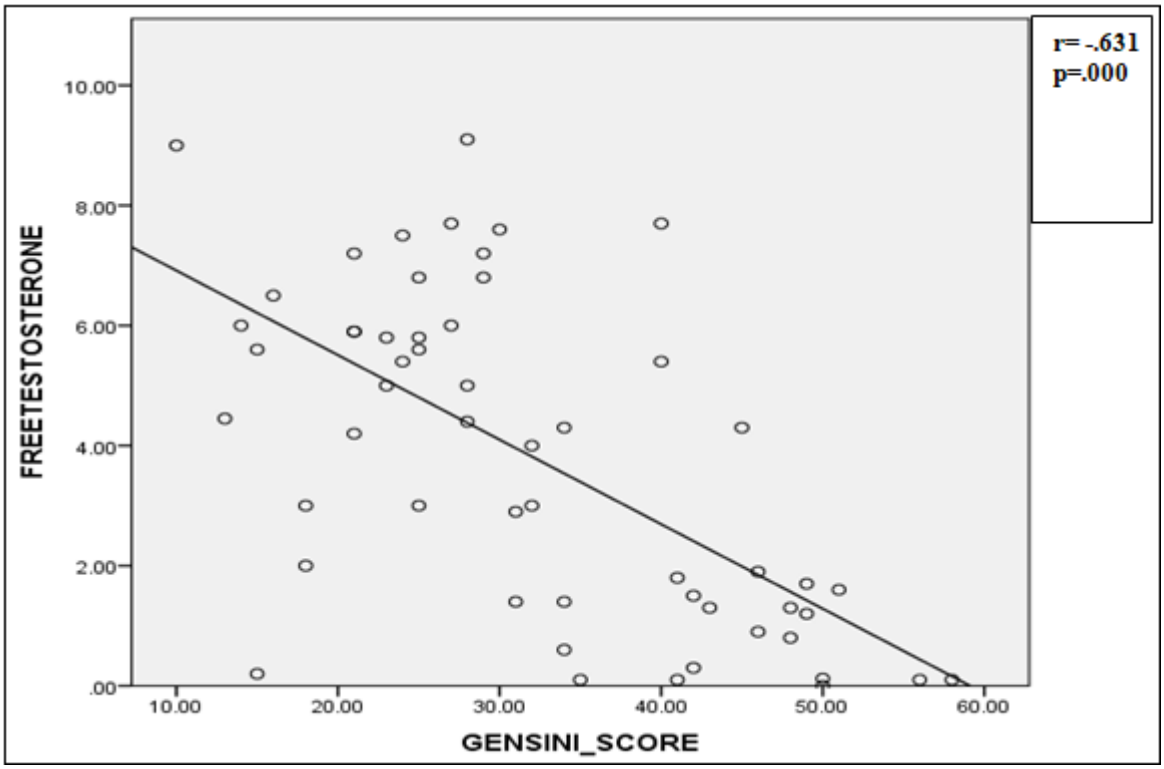

Figure 1: Scattered diagram showing correlation between Gensini score and total testosterone

Correlation between total testosterone level and other variable of interest:

There was significant negative correlation between serum total testosterone levels and Gensini scores (r= - .509, p=0.000). The total testosterone concentrations also negatively correlated significantly with age and BMI (P<0.05), as shown in table - 4.

Similarly, it is seen from figure - 1 that serum free testosterone levels also showed statistically significant negative correlation with Gensini score (r= - .631, p=.000).

Table 4: Multiple linear regression analysis with Gensini score as the dependent variable (N=55).

Variables	β - coefficients	95% confidence interval for B		p - value
		Lower limit	Upper limit	
Age (years)	.601	.426	.803	0.000*
BMI (kg/m^2)	.190	0.042	1.303	0.037*
Diabetes	−.210	−10.146	−.502	0.031*
Hypertension	.101	−1.441	6.833	.196
Smoking	0.071	−2.597	6.461	.395
Alcohol	−0.082	− - 5.894	1.908	.309
Total Testosterone (ng/ml)	−.238	−4.375	−.524	0.014*
Free Testosterone (pg/ml)	−.179	−1.736	.131	0.09

A multiple linear regression analysis with the Gensini score as the dependent variable

In table 4, Gensini score was used as the dependent variable, and the independent variables were age, BMI, diabetes, hypertension, smoking, alcohol intake, total and free testosterone. We showed that age, BMI, diabetes and total testosterone were found to be an independent risk factors for higher Gensini score.

5. Discussion

In this cross - sectional study, Diabetes mellitus and BMI differed significantly among the two study groups (p<0.05). These findings were supported by the study conducted by Fallah N et al[6] and English KM et al[7]. The presence of hyperglycemia favors atherosclerosis and imparts an increased risk of Coronary Artery Disease by non - enzymatic glycosylation of proteins and lipids, collectively known as browning reaction. Early glycosylation products of glucose on long lived proteins (eg. vessel wall collagen) undergoes complex series of chemical rearrangement to form advanced glycosylation end products (AGEs). AGEs accelerate atherosclerotic process via the non - receptor dependent and acceptor mediated mechanisms.[8] Adipose cells, being endocrine in nature, have a pivotal role in body metabolism homeostasis by releasing pro - inflammatory cytokines (IL - 6, CRP, TNF - ∝) and fat related hormones (leptin, adiponectin) which actively leads to atherosclerotic process.[9]

In our study, total and free testosterone levels were significantly lower in CAD groups as compared to the group without CAD (table - 1). These findings were supported by the results of Mali S et al[10] who found that the levels of total and free testosterone levels were significantly lower in CAD group as compared to normal healthy subjects ($p < 0.001$). The mechanism underlying the protective effect of testosterone has been linked to several factors such as decreasing the expression of pro - inflammatory cytokines such as interleukin - 1β (IL - 1β) and Tumor Necrosis Factor - ∝ (TNF - α) and increasing the expression of anti - inflammatory and anti - thrombogenic cytokine IL - 10.[11] At the physiologic levels, it inhibits reactive oxygen species (ROS) formation, however at supraphysiologic level testosterone has opposite effects by reducing nitric oxide (NO) formation and increasing oxidative stress at a cellular level and can lead to mitochondrial dysfunction.[12] These mechanisms give an insight on the relation between low testosterone and inflammation, atherosclerosis and its progress, and cardiovascular mortality.

In the present study, among fifty - five male CAD patients, it was shown that CAD patients with Gensini score >30 had significantly lower total testosterone levels and free testosterone levels than cases whose Gensini score were ≤30 (table - 2). Li L[13] in their study had also reported that the severity of CAD was significantly lower in the groups of higher testosterone (4.383 - 19.002 ng/ml, Gensini score: 16) compared to two other groups of lower testosterone (0.002 - 3.391 ng/ml, Gensini score: 40.5; 3.392 - 4.382 ng/ml, Gensini score: 20). It may be due to the protective effect of endogenous testosterone on cardiovascular vasodilation and increase blood flow in men.[14]Low circulating levels of testosterone might be associated with hypercoagulabilty state because testosterone levels are negatively correlated with factor - VII activity and $\propto_2$ - antiplasmin.[15]
We demonstrated a statistically significant negative correlation between severity of CAD and serum total and free testosterone levels in male patients (table 3 and figure 1). This finding was in agreement with the study done by Li L et al[13] where their findings revealed a negative correlation between Serum testosterone levels and Gensini score ($r= -0.188$, $p= 0.000$). Testosterone deficiency is shown to negatively affect carotid intima media thickness, hence it is rational to presume that it would have the same deleterious effect on the coronary arteries.[16]

In our study, total testosterone had significant negative correlation with age ($r=-0.397$, $p=0.003$) and BMI ($r=-0.324$, $p=0.016$). In the study conducted by Fukui M et al[17], significant inverse correlations were found between serum free testosterone concentration and age ($r=-0.420$, $p< 0.0001$) while the inverse relation between serum total testosterone and age was not significant ($r=-0.099$, $p=0.1295$). There is debate about whether the decline of testosterone with ageing is a natural physiological process or a result of long - term comorbidities and lifestyle decisions. Beattie MC et al, [18] in their rat models study suggested that aging may cause a reduction in the amount of testosterone produced by Leydig cells in response to leutinizing hormone. A typical consequence of testosterone synthesis is reactive oxygen species (ROS), which are produced by the mitochondria of Leydig cells. The DNA of the Leydig cell may be damaged over time by the build - up of ROS, which would prevent the cell from generating testosterone.[18]

The present study demonstrated significant negative association between total testosterone and BMI (table - 3). Testosterone deficiency is associated with increased fat mass (particularly central obesity) and decrease lean mass in males. The mechanisms of testosterone effects on enzymatic pathways of fatty acid metabolism, glucose control, and energy utilisation are evident and frequently tissue specific, with differential effects noted in different regional fat depots, muscle, and liver. Testosterone replacement therapy has shown positive benefits on measures of obesity, which can be largely attributed to both direct metabolic effects on adipose and muscle tissue and also possibly to an increase in enthusiasm, vigour, and energy that enables obese people to lead more active lifestyles.[19]

Our study also showed that the negative association of total testosterone with Gensini score remainedsignificant even after adjustment for well - established traditional risk factors (table - 4). These findings were in agreement with the study done by Gururani K et al[20] and Adamkiewicz M et al[21] who showed low total testosterone to be an independent risk factor for Gensini score or severity of CAD. It is likely to be a complicated web of interrelated processes that include accelerated atherosclerosis, abnormally activated inflammatory responses, decreased vasomotion, and endothelial dysfunction.

The present study shows that there is significant association between testosterone and coronary artery disease suggesting that low testosterone may be one of the causes rather than the consequence of CAD in men. The precise mechanism underlying our findings and its clinical relevance require further elucidation. Also, due to small sample size which is one of the limitation of this study, further studies with larger sample size are necessary to come to an unequivocal conclusion.

6. Conclusion

In conclusion, men with CAD had lower total and free testosterone compared to normal groups without CAD. The findings from the present study provide evidence for a role of testosterone in the pathogenesis of CAD along with traditional risk - factors. This study also suggests low testosterone level as an independent risk factor for CAD. However further study with large population may be required to fully understand the role of testosterone as a modifiable risk factor.

Declarations

Ethics approval and consent to participate

The study was approved by the Research Ethics Board, Institutional Ethics Committee (IEC), Regional Institute of Medical Sciences (RIMS), Imphal (Ref no. – A/206/REB - Comm (SP) /RIMS/2015/676/17/2020). All participants provided written informed consent before being enrolled in the study.

Consent for publication
As part of their written informed consent to participate in the study, subjects also consented to the publication of their anonymized data for research purposes.

Availability of data and materials
Hospital policy prevents public dissemination out of concern for patient privacy.

Competing interest
We declare no competing interest.

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Not applicable

Author's contribution
NA participated in study design, perform biochemical analysis and was a major contributor in drafting the manuscript. LS and TSS conceived the study and assisted in study coordination and manuscript revision. LR, KRD and PM assists in biochemical analysis. All authors read and approved the final manuscript.

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