Sex Hormone Levels – Estradiol, Testosterone, and Sex Hormone Binding Globulin as a Risk Marker for Atherosclerotic Coronary Artery Disease in Post-menopausal Women

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Abstract

Context: Sex hormones levels determine the risk of occurrence of coronary artery disease (CAD) in post-menopausal (PM) women. **Aims:** To investigate the relationship between sex hormones (estradiol and testosterone)/sex hormone binding globulin (SHBG) and cardiovascular risk factors in PM women. In addition, we learned the association between these sex hormones/SHBG and the occurrence of atherosclerotic CAD event in PM women. **Settings and Design:** Cross-sectional case-control study. **Subjects and Methods:** Subjects recruited in the present study were from the cardiology outpatient clinic or Emergency department Guwahati Medical College and Hospital, Assam. The subjects were grouped into two categories after appropriate exclusion criteria: Cases – PM women with documented CAD ($n = 40$) and controls – Healthy PM women ($n = 30$). The medical history, clinical examination, and investigations including serum estradiol, total testosterone, SHBG, free testosterone index (FTI), high-sensitivity C-reactive protein (hs-CRP), lipid profile, carotid intima-media thickness (CIMT), fasting plasma glucose (FPG), and postprandial plasma glucose (PPPG) were done and analyzed. **Statistical Analysis Used:** Pearson correlation between sex hormones and CAD risk factors was done. The association between sex hormones and CAD risk factors among PM women was analyzed by multiple logistic regression. The statistical significance was set at the 0.05 level. **Results:** The mean age of all the subjects was 62.27 ± 6.9 years. Among the cases, a significant positive correlation was found between total testosterone/FTI and waist circumference, W/H ratio, triglyceride levels, hs-CRP, and CIMT ($P < 0.01$). In addition, a significant negative correlation was found between total testosterone and FTI with high-density lipoprotein-cholesterol levels ($P < 0.01$). The multiple logistic regression analysis showed that total testosterone levels ($P < 0.01$) and SHBG ($P < 0.01$) are independently associated with the occurrence of atherosclerotic CAD in PM. **Conclusion:** We conclude that increased serum testosterone levels and low SHBG in PM women are associated with the development of atherosclerotic cardiovascular risk factors.

Keywords: Coronary artery disease, estradiol, post-menopausal women, SHBG, testosterone

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of death among women worldwide.

Studies examining associations between endogenous estrogen levels and CVD risk factors have yielded conflicting results.[1-6] Indirect evidence for a role of androgens comes from findings of clinical studies showing an unfavorable cardiovascular risk profile, relating to imbalance in estradiol to testosterone ratio.[7-10] The present study was planned to analyze the influence of endogenous sex hormones (estradiol and testosterone) and sex hormone binding globulin (SHBG) on the risk factors for coronary artery disease (CAD) in post-menopausal (PM) women.

SUBJECTS AND METHODS

The present study was a cross-sectional, case-control study Gauhati Medical College Hospital, Guwahati over a period from December 2015 to December 2017. The study was approved by the institutional ethical committee, and informed consent was taken from every subject or their guardian.

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The study subjects were divided into two groups. Cases that included PM women of age between 50 and 75 years attending the Cardiology outpatient department/Emergency department of Gauhati Medical College Hospital with CAD (Atherosclerotic) defined as subjects who have had documented myocardial infarction as per clinical presentation, Electrocardiogram, and elevated cardiac enzymes, or subjects who have a documented angiography showing 70% occlusion of any of the major coronary vessels not on prior treatment. The controls were age, gender, and body mass index (BMI) matched healthy PM women. The PM status was defined as cessation of menses for more than 12 months in presence of natural menopause. CAD was excluded among controls by symptom analysis (angina or angina equivalent), physical examination, ECG, and chest X -ray. The patients with history of endocrine diseases, hepatic disease, renal failure, diabetes mellitus or on statins or hormonal replacement medications, smokers, alcoholism, and who have undergone oophorectomy were excluded from the study. The patients who fulfilled the study criteria underwent a detailed medical history and clinical examination.

Risk factors for atherosclerotic CAD as defined by metabolic syndrome criteria International Diabetes Federation (IDF) criteria for Asian population.[10] The cut-off for Asian population are summed below:

- Waist circumference (WC) >80 cm in Asian population women
- BMI >23 (Asian cut-off for obesity)
- Waist and hip circumference ratio >0.8 women suggestive of android obesity.

All subjects underwent detailed history and thorough physical examination. Weight, height, BMI, and waist and hip circumference were measured with standard techniques. Blood sample for high-sensitivity C-reactive protein (hs-CRP) and lipid profile was taken from cases from the emergency department patients once they were diagnosed to have myocardial infarction irrespective of the time of presentation before starting high doses of statins or aspirin or heparin. For those patients with documented CAD angiographically not on medications, blood samples were taken in fasting state. Venipunctures for hormonal assay (estradiol, total testosterone, and SHBG) were performed in the morning after subjects had fasted for at least 8 h. The blood samples were subjected to biochemical and hormonal assay. The samples were immediately centrifuged, and the serum was stored at −20°C until hormonal assayed. The subjects were designated to have metabolic syndrome as per IDF criteria.

Assay of hormones
Serum estradiol was estimated by Elecsys Estradiol II assay (ROCHE COBAS) that employs a competitive test principle using a polyclonal antibody specifically directed against 17β-estradiol. Intra-assay and inter-assay coefficient of variation varies from 5.6 to 6.5% to 3.1 to 5.0%. The testosterone assay (ROCHE COBAS) was also according

to a competitive test principle using a monoclonal antibody specifically directed against testosterone. Intra-assay and inter-assay coefficient of variation varies from 5.2 to 6.8% to 3 to 4.5%. The SHBG assay (ROCHE COBAS) employed sandwich assay using two monoclonal antibodies specifically directed against human SHBG. Intra-assay and inter-assay coefficient of variation varies from 2.1 to 2.7% to 2.5 to 4% for SHBG.

Assay of biochemical markers
Fasting plasma glucose (FPG) and postprandial plasma glucose (PPPG) were measured using glucose oxidase method, and lipid profile was measured by enzymatic colorimetry, using Vitros 5600 automated analyzer. Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald formula. Non-high-density lipoprotein cholesterol (HDL-C), calculated as the difference between total cholesterol and HDL-C, has been documented to be equivalent to LDL-C in predicting CVD. Triglyceride (TG) to HDL-C ratio was calculated as an additional predictor of CVD risks. The hs-CRP was measured from stored serum samples using a solid phase chemiluminescent assay with the immilite 1000 (Siemens Heathcare Diagnostics) and intra-assay coefficient of variation of 5.0%. The cardiovascular risk assessment cut-offs for hs-CRP have been recommended by American Heart Association (AHA) as low risk: (<1.0 mg/L), average risk: (1.0 ~ 3.0 mg/L), and high risk: (>3.0 mg/L).[11]

Carotid intima media thickness (CIMT) was estimated in both cases and control by an Advanced Technology Laboratories High Definition Imaging (ATL HDI) 1500 ultrasound system (B-mode ultrasound) using a 10–12 MHz linear transducer (Siemens), which is considered as a accepted surrogate marker of atherosclerosis. In healthy middle-aged adults, CIMT values between 0.6 and 0.7 mm have been considered normal, whereas CIMT of 1 mm or more has been associated with significant increased absolute risk of CHD.[12] The measurement of CIMT varies with age and values >0.8 mm are considered abnormal in younger population and confer increased absolute risk of CHD.[13]

Results

As seen in Table 1, the mean age of the cases and control were 62.27 ± 6.81 and 63.03 ± 5.24 years, respectively (P = 0.49). There were no significant differences between the mean BMI, blood pressures, and serum estradiol levels among the cases and controls. However, the cases had significant higher mean WC, waist/hip (W/H) ratio, total cholesterol, TGs, non-HDL-C total testosterone, free testosterone index (FTI), estradiol/testosterone (E2/T) ratio, hs-CRP, and CIMT. There was also a significant low HDL-C and SHBG levels among the cases in comparison to controls.

Discussion

The relationship between estrogen and coronary heart disease has been widely described. Estrogen has been known to have

Table 1: Baseline characteristics of cases (*n*=40) and controls (*n*=30)

Variable	Cases (*n*=40) Mean±SD	Control (*n*=30) Mean±SD	*P*
Age (years)	62.27±6.81	63.03±5.24	0.49
BMI (kg/m²)	25.09±3.98	25.36±4.1	0.55
WC (cm)	88±6.2	82±5.8	<0.01
WHR	0.82±0.06	0.77±0.08	<0.01
SBP (mm Hg)	120.65±8.2	120.38±10.1	0.33
DBP (mm Hg)	86.5±10.14	82.1±4.2	0.26
Total cholesterol (mg/dl)	199.55±40.1	190.03±22.8	0.04
LDL-C (mg/dl)	124.77±38.9	129.65±28.11	0.11
HDL-C (mg/dl)	35.75±8.95	40.34±9.12	<0.001
TG (mg/dl)	215.5±94.29	122.92±50.1	<0.01
Estradiol (pg/ml)	11.02±2.05	11.23±2.42	0.7
Total testotterone (ng/dl)	18.72±3.98	12.53±2.64	<0.01
E2/T ratio	0.12±0.16	0.11±0.08	0.03
SHBG (mcg/ml)	3.21±0.38	3.85±0.39	<0.01
FPG (mg/dl)	74.52±10.32	78.32±8.2	0.12
PPPG (mg/dl)	121.75±11.38	124±9.26	0.45
hs-CRP (mg/L)	5.68±3.39	2.70±0.9	<0.01
CIMT (mm)	0.78±0.10	0.66±0.09	<0.01
Free testosterone index (%)	2.38±0.83	2.11±0.69	<0.01
Non-HDLC (mg/dl)	164.8±18.3	150.4±14.2	<0.01

WC: Waist circumference, WHR: Waist hip ratio, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, Non-HDL-C: Non-high-density lipoprotein cholesterol, TG: Triglyceride, E2/T: Estradiol/Testosterone ratio, SHBG: Sex hormone binding globulin, FPG: Fasting plasma glucose, PPPG: Postprandial plasma glucose, hs-CRP: High-sensitivity C-reactive protein, CIMT: Carotid intima media thickness

anti-inflammatory and anti-oxidative activity. These translates to beneficial effects on vascular and lipids metabolism. From the baseline characteristics, we found that all PM women (both case and control) had a higher mean BMI (cases 25.09 ± 3.98 and controls 25.36 ± 4.1) and higher mean Waist circumference (cases 88 ± 6.2 Centimeter and control 82.1 ± 4.2). The mean BMI in both populations fell in the obesity range, and mean WC values in these PM women were more than the cut-off placed for Asian as per IDF criteria.[10] Moreover, among the cases the W/H ratio were above 0.8 that again defines metabolic syndrome in women. The WC and W/H ratios are clinical markers of central adiposity and known to be associated with CAD.[14,15] Earlier studies have shown that menopause state is a relative change in body composition, particularly fat mass.[16,17] The studies of the fat topology suggest that there may be increased likelihood of a menopause effect owing to hypoestrogenemia.[18,19] Further, these risks take on new relevance as current studies increasingly identifies that adipose tissue is an endocrine organ, secreting a wide variety of cytokines including leptin, adiponectin, resistin, plasminogen activator inhibitor, tumor necrosis factor-α, and interleukin (IL)-6 with immunological, vascular, and metabolic actions.[20] All these inflammatory markers may increase the risk of coronary events among the menopausal women.[21]

Among the baseline lipoprotein profile, the mean HDL-C was 35.75 ± 8.95 and 40.34 ± 9.12 mg/dl in the cases and controls, which is far below the desired level. We also found that the cases had higher serum TGs and lower HDL-C than that in control groups (*P* < 0.01). These findings were similar to both the Los Angeles atherosclerosis study and the Study of Women's Health Across the Nation Heart women (SWAN) heart women, which demonstrated that the antiatherogenic effect of HDL diminishes in women around the age of menopause,[22,23] and it was suggested that it is possibly related to changes in the lipoprotein sub-class profile observed during the menopausal transition. From these initial results, we can firmly infer that the PM state is a state of metabolic syndrome where the body composition and lipid profile changes Indian studies in pre- and PM women has found that the prevalence of metabolic syndrome had varied from 31% to 60% in different regions.[24] The prevalence of metabolic syndrome in north India was 45% in premenopausal and 55% in PM women according to various studies.[25] The components of metabolic syndrome in Indian women that was most prevalent among the subjects having metabolic syndrome was abnormal WC (94%) followed by hypertension (71.14%), low-HDL (55.14), abnormal TG (40%), and diabetes (35.71%).[26,27] The prevalence of metabolic syndrome among PM women was significantly higher (*P* < 0.001) than that in premenopausal women as per IDF criteria (premenopausal 45% and PM 55%). In our study, we found 47% patients with abnormal WC, 91% low HDL-C, 47% high TG, and 8% hypertension among all the subjects including cases and controls. Out of those, metabolic syndrome was seen in 60% cases and 16% of the controls as IDF criteria.

Sex hormone levels showed a comparable estradiol levels among the both groups with significant elevated total testosterone, FTI, and E2/T ratios. The SHBG levels were lower among the cases (*P* < 0.01). hs-CRP and CIMT are considered as peripheral markers of atherosclerosis in cardiovascular system were elevated among the cases (*P* < 0.01). Several studies in PM women have shown a positive correlation between serum testosterone levels and occurrences of CAD.[4-13] In the current study, we found total testosterone levels and FTI are related with central adiposity as evident from its significant association with WC and W/H ratio. Although the relationship between testosterone on body composition in women remains controversial, it has been shown that serum level of estradiol and testosterone possibly affects body fat distribution in PM women. Söderberg *et al*. in his study has elaborated a similar finding to ours.[28] The study characterized the relationship between biologically active testosterone and leptin after careful stratification for gender and adiposity among the men and pre- and PM women. The study showed the women (both pre-menopausal and PM) with increased central obesity (as evident by increased W/H ratio and WC) had a positive correlation with serum total testosterone levels (*r* = 0.59, *P* < 0.01).[28] Few other studies also has supported similar association among PM women

suggesting abdominal adiposity is associated with a relatively more androgenic sex hormone profile.[29-31] It is well-known that central distribution of adiposity such as WC and waist to hip ratio (WHR) show stronger associations with CVD and its CVD risk factors.[14,15] Phillips *et al*.[32] reported a positive correlation between free testosterone and visceral fat mass (visceral fat mass in the abdominal cavity was assessed by Computerized Tomography) in healthy PM women. Two studies reported an inverse association between SHBG and visceral fat tissue.[33,34] In another study, including pre-, peri-, and PM women, high Bioavailable testosterone, and low SHBG levels were also found to be associated with an increase in visceral fat, independently of age, insulin resistance, and estradiol.

In the present study, we found a correlation and association between total testosterone (and FTI), estradiol levels, and SHBG with lipoprotein profiles among the cases. We found that the elevated levels of TGs among cases and correlated with high testosterone and low estradiol levels among the cases [Tables 2 and 3]. Such relations were not seen with LDL-C or total cholesterol. However, our study also revealed HDL-C levels were positively dependent on SHBG concentrations [Tables 4 and 5] and negatively related to total testosterone levels. The relationship seen in the above study is similar to the lipid profile seen among women with hyperandrogenic polycystic ovary syndrome. They have an abnormal lipid profile, characterized by elevated TG and reduced HDL-C levels.[35,36] Moreover, in female to male transsexuals, testosterone administration has been associated with a reduction in HDL-C and an increase of TG levels.[37] In a study with obese PM women, the administration of nandrolone decanoate[38] causes a decrease in HDL-C (purpose of this study was to assess the effects on fat distribution of administering nandrolone decanoate in obese PM women). The mechanisms explained through which testosterone and SHBG affect lipid metabolism are not completely understood, although direct regulatory effects on hepatic lipase (HL) and lipoprotein lipases (LPLs) have been reported. HL and LPL are key enzymes involved in the regulation of TG and HDL-C levels. The LPL activity causes a decrease in TG and an increase in HDL-C levels, whereas HL activity is associated with a decrease in HDL-C. The sensitivity of lipolytic enzymes for androgens is further supported by findings from the HERITAGE study showing a strong inverse association between SHBG and HL activity and a positive association between SHBG and LPL activity.[39] The study included subjects of both sexes between 17 and 64 year. In this study, the hypotheses were that there are significant associations between SHBG, sex steroid hormone levels, and post-heparin lipolytic enzyme activities, and that these associations are independent of concomitant variation in adiposity and the metabolic profile. In women of the present study, after statistical adjustment for fasting insulin and adiposity measures, the negative association between HL and SHBG level did not change. Thus, the regulation of HL by free androgens and/or estrogens may be presumably independent from concomitant variations in insulin levels or abdominal

Table 2: Distribution of various components of metabolic syndrome in the study population (*n*=70)

Components of metabolic syndrome	Percentage (%) of patients (*n*=70)
Abnormal waist circumference (cm)	47% (*n*=33)
Low HDL-C (mg/dl)	91% (*n*=63)
High triglycerides (mg/dl)	47% (*n*=33)
Systemic hypertension (mm Hg)	8% (*n*=6)

Table 3: Correlation between total testosterone levels and free testosterone index with lipoprotein profile/waist circumference/W/H ratio/CIMT, and hs-CRP among the cases (*n*=40)

Pearson correlation coefficients, *n*=40 (*P*)	
	Total testotsterone and free testosterone index
Cholesterol	r1=0.16, r2=0.09, *P*=0.38, *P* =0.48
LDL -C	r1=008, r2=0.04, *P*=0.58, *P*=0.6
Triglyceride	r1=0.64, r2=0.84, *P*<0.01, *P*<0.01
HDL -C	r1=−0.29, r2=−0.36, *P*=0.04, *P*=0.02
Waist circumference	r1=0.58, r2= 0.8, *P*<0.01, *P*<0.01
Waist/Hip (W/H) ratio	r1=0.48, r2=0.30, *P*=0.01, *P*=0.03
CIMT	r1=0.78, r2=0.91, *P*<0.01, *P*<0.01
Hs-CRP	r1=0.68, r2=0.80, *P*<0.01, *P*<0.01
Non-HDL-C	r1=0.48, r2=0.50, *P*<0.01, *P*<0.01

r1: Total testosterone correlation, r2: Free testosterone index correlation. Among the post-menopausal women with CAD, a significant positive correlation was found between total testosterone and free testosterone index with waist circumference, W/H ratio, serum triglyceride levels, Non-HDL-C, hs-CRP, and CIMT. The negative correlation of significance was found between total testosterone and free testosterone index with HDL-C levels. The linear regression analysis showed association between total testosterone and free testosterone index with waist circumference, W/H ratio, serum triglyceride levels, Non-HDL-C, hsCRP, and CIMT. (*P*<0.01). No such correlations were seen among the controls

Table 4: Correlation between estradiol levels and lipoprotein profile/Waist circumference/W/H ratio/CIMT and hsCRP among the cases (*n*=40)

Pearson correlation coefficients, *n*=40 (*P*)	
	Estradiol
Cholesterol	0.01, *P*=0.48
LDL-C	0.07, *P* = 0.34
Triglyceride	−0.39, *P*<0.01
HDL-C	0.02, *P* = 0.7
Waist circumference	0.08, *P* = 0.4
Waist/Hip (W/H) ratio	0.02, *P* = 0.3
CIMT	0.11, *P* = 0.91
hs-CRP	−0.64, *P*<0.01
Non-HDL-C	0.2, *P*=0.6

Among the post-menopausal women with CAD, a significant negative correlation was found between estradiol levels and serum triglyceride levels with hs-CRP. The linear regression analysis showed association between estradiol levels and serum triglyceride levels with hs-CRP (*P*<0.01). No such correlations were seen among the controls

Table 5: Correlation between SHBG levels and lipoprotein profile/waist circumference/W/H ratio/CIMT and hs-CRP among the cases (*n*=40)

Pearson correlation coefficients, *n*=40 (*P*)	
	SHBG levels
Cholesterol	0.04, *P*=0.58
LDL-C	0.04, *P*=0.44
Triglyceride	0.20, *P*=0.1
HDL-C	0.32, *P*=0.03
Waist circumference	0.18, *P*=0.64
Waist/Hip (W/H) ratio	0.07, *P*=0.33
CIMT	0.01, *P*=0.61
hs-CRP	0.06, *P*=0.31
Non-HDL-C	0.05, *P*=0.51

Among the post-menopausal women with CAD, a significant positive correlation was found between SHBG and HDL-C levels. The linear regression analysis showed association between SHBG and HDL-C levels ($P<0.01$). No such correlations were seen among the controls

Table 6: Multi-variance logistic analysis (*n*=70) showing the odds of occurrence of an atherosclerotic CAD event in PM women after adjusting for age, BMI, systemic hypertension, lipid profile, and hs-CRP levels

	Variables	**Odds ratio (OR)**	**95% Confidence interval (CI)**	***P***
Model	Estradiol	0.23	0.34-0.92	0.7
Multiple logistic regression	Testotserone	6.76	2.385-19.170	<0.01
	SHBG	−0.82	0.74-0.98	<0.01

The multiple logistic regression analysis showed that total testosterone levels [OR 6.76 (CI -2.34-19.42) $P<0.01$] and SHBG [OR -0.825 (CI-0.74-0.91), $P<0.01$] are independently association with the occurrence of atherosclerotic CAD in PM women

adiposity. Apart from direct regulatory effects, testosterone and SHBG may also influence lipid metabolism indirectly through their associations with obesity. Yasui *et al.*[40] found that associations of SHBG with HDL-C and TGs were no longer significant after controlling for BMI. In another study, a similar lack of independence was observed for the association with HDL-C.[41] These studies may relate the occurrence of harmful lipid pattern in PM women may be also indirectly related to obesity levels. Serum TG levels and SHBG were not altered among the controls.[42] One limitation of interpreting the serum TG levels in our study is that the TG levels were not estimated in fasting state. When patients presented with acute coronary event, considering the alteration of lipid profile that may occur after giving high dose statin for Myocardial infarction blood samples were drawn random. Hence, the levels of TGs in our study may not be accurate. To overcome this limitation, we calculated non-HDL-C levels whose calculation is independent of fasting state. We found that the cases had a higher non-HDL-C levels (164.8 ± 18.3 mg/dl) than that in control groups (150.4 ± 14.2 mg/dl). It is well-known that the relative risk of cardiovascular event is 47% and 143% when

the non-HDL-C is between 160 and 190 mg/dl and more than 220 mg/dl in women. In addition, total testosterone levels positively correlated with non-HDL-C.[24] Relating this, we can explain that the altered estrogen to testosterone ratio in PM women may contribute occurrence in central obesity and altered lipid profile that can itself be a risk factor for future CAD in PM women.

The present study also showed that mean hs-CRP levels were significantly higher among the cases than that in controls group (cases 5.68 ± 3.39 vs. control 2.70 ± 0.9). The estradiol levels negatively correlated with hs-CRP levels among the cases. Similar finding were also found in few studies where a significant differences in the levels of hs-CRP were found regarding pre- and PM women with approximately 3-fold increase of hs-CRP in the serum of PM healthy women when compared to pre-menopausal women.[25] An elevated level of hs-CRP in PM is due to estrogen shortage.[26] Apparently, besides being directly involving in low-grade chronic systemic inflammation, hs-CRP is emerging as the strongest and most independent predictive risk factor for atherosclerosis and CVD.[27] The cardiovascular risk assessment cut-offs for hs-CRP have been recommended by AHA 2004 as low risk: (<1.0 mg/L), average risk: (1.0 ~ 3.0 mg/L), and high risk: (>3.0 mg/L).[11] In our study, the cardiovascular risk assessment from hs-CRP was found to be high in cases and average risk in controls. It is known that hs-CRP binds to oxidized LDL which leads to an increase in adhesion molecules promoting complement proteins and trigger inflammation in atherosclerotic plaques. Furthermore, CRP promote the induction of tissue factor, the remarkable factors on monocyte surface which is considered to be one of the important coagulation factors, also increase adhesion molecules and manipulate the production of nitric oxide.[27] The removal of the regulatory effect of estrogens up on hs-CRP leads to an increase of hs-CRP level in PM women accumulating the increased risk of CVD. It has to be understood that the menopausal state itself is a cardiovascular risk, and women who have an elevated testosterone levels among the menopausal state will further add a risk as evident from our study population. In this study, we were able to find a relationship between increased total testosterone levels and FTI with elevated hs-CRP levels.

We found a significant positive correlation between CIMT and testosterone levels in the cases but not in the controls. The measurement of CIMT is an accepted surrogate marker of atherosclerosis. Ouyang *et al.*[43] in his study focused on a population of PM women without clinically evident CVD and found that high total testosterone and bioavailable testosterone levels were associated with carotid atherosclerosis. Total and bioavailable testosterones were positively associated with common CIMT independent of age, BMI, hypertension, smoking, HDL-C, LDL-C, and insulin sensitivity.[44,45] We can make a strong comment from above study that in PM women, testosterone may be independently associated with greater common CIMT and increases the risk of cardiovascular events in PM women. Further, elevated hs-CRP and altered lipoprotein

levels as seen in our study *per se* can also alter the caliber of vessels.

Finally, we did multi-variance logistic analysis to find the role of estradiol, testosterone, and SHBG independently in the occurrence of CAD event in PM women [Table 6]. After adjusting for age, BMI, blood pressure, lipid profile, and hs-CRP, we found that total testosterone levels [OR 6.76 (CI -2.34-19.42) $P < 0.01$] and SHBG [OR –0.825 (CI -0.74-0.91), $P < 0.01$] are independently associated with the occurrence of atherosclerotic CAD in PM women.

We would like to emphasis that Freudwald index may not be useful if TG levels are above 400 mg%. In our study, none of the patient had Triglyceride above this value. An equal number of case and controls are always considered better, but because of the paucity of study period, we could not attain an equal number that may be a limitation of our study.

Conclusion

The PM state itself is considered a risk factor for CAD. There is a relative change in body composition particularly the fat mass of centripetal distribution is owing to the hypostrogenemia in the menopausal state. Moreover, hs-CRP levels were higher in all patients with menopause. This central obesity is involved in multiple metabolic events that proceed to the occurrence of CAD. Higher serum testosterone levels with low SHBG and comparable serum estradiol levels were seen in the cases. This characteristic sex hormone profile seen in cases were also found to alter the lipoprotein pattern, and further, raise inflammatory markers such as hs-CRP which are risk factors for CAD. The higher chances of a coronary artery event were further demonstrated by an elevation of CIMT as seen among the cases, which is considered as a peripheral marker of atherosclerosis. It was also found that testosterone and SHBG independently associated with the occurrence of atherosclerotic CAD in PM women.

Hence, we can conclude that high testosterone levels and low SHBG directly or indirectly by altering lipid profile and inflammatory markers increase the risk of atherosclerotic CAD among the menopausal women.

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Conflicts of interest
There are no conflicts of interest.

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