

## A study of association of sex hormone milieu with lipid profile for assessment of cardiovascular risk in adult women

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### Abstract

**Introduction:** Women continue to have a high cardiovascular mortality than men in the past 20 years which is underestimated. Cardiovascular disease is clearly polyfactorial, and data on endogenous hormones may improve our prediction of risk. The endocrine environment in women is complex and changes with chronology. So this study becomes a need in women of middle age with objectives-

1. To analyse the level and association of serum Estradiol, Testosterone, SHBG, DHEA with lipid profile.
2. To assess the cardiovascular risk in adult women with respect to their androgenic status.

**Materials and Method:** A cross-sectional study from 120 volunteered adult women of age between 20-40years selected and blood collected for biochemical assay of serum Estradiol, Testosterone, Steroid Hormone Binding Globulin, Dehydroepiandrosterone and lipid profile by standard methods. All quantitative data obtained were analysed with a set statistical significance at  $p < 0.05$ .

**Results:** In our study mean age of adult women was  $29.05 \pm 5.8$  years. The mean of sex hormone profile were in normal range. Testosterone levels even in normal range showed a positive association with LDL-C ( $r=0.392$ ,  $p < 0.05$ ), Atherogenic Index of Plasma ( $r=0.452$ ,  $p < 0.05$ ) and negative association with HDL-C ( $r=-0.114$ ,  $p=0.21$ ).

**Conclusion:** The study shows in adult women as the age advances the androgen levels influences dyslipidemia and cardiovascular risk.

**Keywords:** Cardiovascular disease, Dehydroepiandrosterone, Estrogen, Steroid Hormone Binding Globulin, Adult women, Testosterone

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### Introduction

Cardiovascular diseases (CVD) are the number one leading cause of death globally. Women continue to have a high cardiovascular mortality than men in the past 20 years which is underestimated.<sup>(1)</sup> Coronary Artery Disease (CAD) is clearly polyfactorial, and data on endogenous hormones may improve our prediction of CAD.<sup>(2)</sup> The female sex hormone milieu oestrogen, testosterone, Dehydroepiandrosterone (DHEA) occurs with an increase in cardiovascular risk factors including changes in lipids and body fat content.<sup>(3,4)</sup> The relationship between hyperandrogenemia and endothelial function, lipid profile, insulin resistance, inflammatory responses, altered vascular smooth muscle reactivity, and hypertension are established and thus it contributes to the onset and progression of CVD.<sup>(5)</sup>

Recent data from the National Health and Nutrition Examination Surveys (NHANES) have shown that over the past two decades the prevalence of myocardial infarctions has increased in midlife (35 to 54 years) women, while declining in similarly aged men.<sup>(6)</sup>

The endocrine environment in women is complex and changes with chronology and menstrual cycle. Much interest and research in the last 15 years has focused on the oestrogenic effects on the risk and

progression of cardiovascular disease.<sup>(7)</sup> The Heart and Estrogen/Progestin Replacement Study found that there was no benefit to hormone replacement in women with established CAD and an increase of coronary heart disease events was found in the first year of the trial.<sup>(3)</sup> In the Women's Ischemia Syndrome Evaluation study it was shown that young women with endogenous oestrogen deficiency have a more than sevenfold increase in coronary artery risk.<sup>(8)</sup> Thus the effect of the sex hormones on the aging cardiovascular system is unclear. Therefore a study of multiple cardiovascular risk factors in middle age of life of women gains significant.

### Aims and Objectives

1. To analyse the level and association of serum Estradiol, Testosterone, Steroid Hormone Binding Globulin, Dehydroepiandrosterone and lipid profile in adult women.
2. To assess the cardiovascular risk in adult women with respect to their androgenic status.

### Materials and Method

The research work was conducted in 120 volunteered adult women as a cross-sectional study.

**Inclusion Criteria:** Volunteer women of age greater

than 20 or less than 40years with at least nine menstrual cycles in 12-month period. Exclusion criteria: Women with Polycystic Ovarian Disease, gynecological malignancies, IHD, hypertension, DM, History of liver, thyroid or renal diseases. Patients on treatment with OCP, infertility treatment, steroid, hypolipidemic, anti-inflammatory and thyroid drugs, Women with smoking/alcoholic habits, Pregnant and breast feeding women.

For all the study subjects detailed clinical history, menstrual history, family history of CVDs, general and gynecological examination with their blood pressure and anthropometric measurement (weight, height) was recorded in a protocol format taking the informed consent.

5 ml of fasting blood sample on 2-5<sup>th</sup> day of LMP was collected under aseptic condition in a clean dry test tube and allowed to clot. After the retraction of the clot, the sample centrifuged and serum separated was used for the following biochemical parameters given below-

1. Serum Estradiol,<sup>(9)</sup> Testosterone,<sup>(10)</sup> Steroid Hormone Binding Globulin<sup>(11)</sup> (SHBG), DHEA<sup>(12)</sup> – Enzyme immunoassay for all samples were performed using the DRG Diagnostics kit by plotting the standard curve with a ELISA reader
2. Other parameters Serum Total Cholesterol(TC) (CHOD-PAP Method), Triglyceride (TGL) (GPO – PAP Method), HDL- Cholesterol (Precipitating Reagent Method), Glucose (Glucose oxidase – Peroxidase Method), Total protein(Biuret method) and serum Albumin (Bromocresol green method) were analysed using kits obtained from Primal Health Care in semi-auto analyzer.
3. Calculated parameters: Body Mass Index (BMI) = Weight (Kg)/Height (m<sup>2</sup>).

Atherogenic Index of Plasma(AIP) -  $\log(\text{triglycerides (mmol/l)/HDL-C(mmol/l)})$ .<sup>(13)</sup>

**Statistical Analysis:** All quantitative data obtained were presented as mean  $\pm$  SD, students 't' test and qualitative data as %. Correlation analysis will be performed with a set statistical significance at  $p < 0.05$ .

## Results and Discussion

A total of 120 volunteered normally menstruating women were included in this study and were categorized to Group I (20-30years) and Group II ((31-40years) shown in Table 1 The mean age of adult

women was  $29.05 \pm 5.8$  years compared to  $38 \pm 7$  years in Sower et al.<sup>(14)</sup> All the women had their blood pressure and other basic parameters within normal limits.

**Table 1: Age distribution of study subjects**

Group (years)	Total women (N=120)	Age (years) Mean $\pm$ SD	Menstrual cycle(days) Mean
Group I (20-30)	61	24.0 $\pm$ 2.5	4/30
Group II (31-40)	59	34.2 $\pm$ 3.06	4/28

The mean of sex hormone profile were in normal range for the age. The mean Estradiol, Testosterone, SHBG showed increased level in Group II (31-40years) compared to the Group I (20-30years). The mean DHEA of Group II (31-40years) showed decreased level compared to Group I (20-30years) and statistically not significant.

Oestrogen is one of the important female sex hormone which has a role in lipid metabolism. The research in the last 15 years has focused on the oestrogenic effects on the risk and progression of cardiovascular disease.<sup>(7)</sup> The Estradiol of study subjects was  $62.55 \pm 38$ (reference value 13-191pg/ml) with range of 44.04-252.7pg/ml and showed in normal limits in the categorized groups in Table 2. The Estrogen changes the vascular permeability by increasing nitrous oxide production, maintains a healthy lipoprotein profile, stabilizes the endothelial cells, enhances antioxidant effect, and alters fibrinolysis protein thus promoting cardioprotective mechanisms.<sup>(2)</sup> The role and relative importance of Testosterone to women's health in reproductive years, the menopause transition, and the postmenopause has been a matter of speculation.<sup>(15)</sup> The mean Testosterone value of Group II(31-40yrs) was  $1.47 \pm 1.0$ (reference value 0.26-1.22ng/ml) observed to be increased compared to Group I(20-30years). Hypoandrogenemia in men and hyperandrogenemia in women are associated with increased risk of CAD but also with visceral obesity low HDL-C, elevated TGL, LDL-C and Plasminogen Activator Inhibitor.<sup>(16)</sup>

**Table 2: Body mass Index, Sex hormones and lipid profile of study subjects**

Parameters (Normal Reference range)	Group I (N=61) Mean $\pm$ SD	Group II(N=59) Mean $\pm$ SD	t-value (p-value)
Estradiol (13-191pg/ml)	58.75 $\pm$ 37.7	66.41 $\pm$ 39.5	1.086 (0.27)
Testosterone (0.26-1.22ng/ml)	1.08 $\pm$ 0.8	1.47 $\pm$ 1.0	1.41 (0.161)
DHEA (1.3-9.8ng/ml)	12.04 $\pm$ 6.9	10.6 $\pm$ 7.9	1.064 (0.289)
SHBG (15-120nmol/l)	170.37 $\pm$ 58.07	180.7 $\pm$ 77.6	0.77 (0.442)
T.Cholesterol (150-	175.53 $\pm$ 24.7	180.27 $\pm$ 27.7	0.996(0.321)

200mg/dl)			
Triglyceride (<150mg/dl)	141.18 ± 13.2	153.03 ± 37.3	2.33(0.02)*
HDL-C (45-65mg/dl)	48.7 ± 3.8	48.4 ± 3.9	0.42(0.675)
LDL-C (< 100 mg/dl)	98.56 ± 28.2	101.25 ± 24.7	0.55(0.583)
AIP (<0.11 low risk)	0.26± 0.05	0.25± 0.05	0.27(0.783)
BMI (<23kg/m <sup>2</sup> )	22.86 ± 2.5	22.4 ± 2.1	3.2(0.001)*

(\* p value <0.05 and statistically significant)

Table 2 shows that the Estradiol is within normal limits for age. SHBG levels are elevated in both groups. Mean BMI is also normal. The Testosterone and Triglycerides were mildly increased in the Group II (31-40years). Hypertriglyceredemia in Group II showed to be statistically significant.

Mean value of lipid profile were in normal range. On the categorized groups, the mean Triglyceride (153.03 ± 37.3 mg/dl) was increased in Group II (31-40years) and significant as shown in Table 2. Hypertriglyceredemia >150mg/dl was 37% among Group II (31-40yrs), whereas only 23% in Group I (20-30yrs) women. 31.6% (38/120) were obese with BMI>23 kg/m<sup>2</sup> but their mean BMI 22.8 ± 2.5 in Group I(20-30) and 22.4± 2.1 kg/m<sup>2</sup> in Group II(31-40 years) (refer Table-2). BMI ranged from 16.2 – 28.1 kg/m<sup>2</sup> in this study. So the study subjects had less BMI compared to the mean BMI of in Sowers et al<sup>(19)</sup> 25.5 ± 2.4 kg/m<sup>2</sup> and in Mary Fran R et al<sup>(17)</sup> 27.2 ± 0.6 kg/m<sup>2</sup> studies. Hypertriglyceredemia was present in 30% (36/120) and 31.6% (38/120) were obese women as shown in Fig. 1.

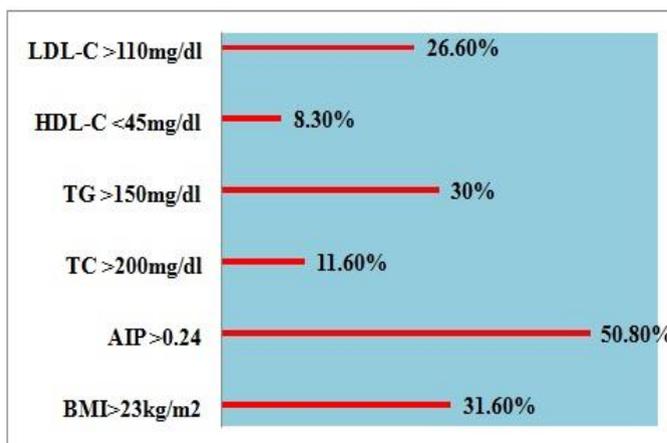


Fig. 1: Distribution of cardiovascular risk profile among study subjects

Table 3: Correlation analysis of sex hormones with lipid profile

Parameters	Estradiol (pg/ml) r-value	Testosterone (ng/ml) r-value	DHEA (ng/ml) r-value	SHBG (nmol/l) r-value
Age (years)	0.127(0.16)	0.164(0.07)	-0.197*(0.03)	-0.059(0.52)
BMI ((kg/m <sup>2</sup> )	-0.002(0.98)	0.079(0.38)	0.128(0.16)	-0.186(0.041)
T.Cholesterol (mg/dl)	-0.013(0.88)	0.262 (0.003)	-0.141(0.12)	0.105(0.25)
Triglyceride (mg/dl)	-0.06(0.51)	0.208 (0.02)	-0.06(0.51)	-0.045(0.62)
HDL-C (mg/dl)	0.129(0.15)	-0.114(0.21)	0.065(0.47)	-0.003(0.97)
LDL-C (mg/dl)	0.007(0.93)	0.392* (0.001)	-0.144(0.11)	0.121(0.18)
AIP	-0.037(0.68)	0.452*(0.0001)	0.102(0.26)	-0.128(0.16)

(\* p value <0.05 and statistically significant)

Table 3 Correlation analysis of Testosterone was positive with age, TC, TGL, LDL-C, AIP and negative with HDL-C. DHEA showed negative association with age.

The correlation analysis of Estradiol showed no statistical significant association with lipid parameters. Estradiol had positive association with HDL-C (r= 0.129) which is accepted with the hypothesis proposed that higher HDL and lower LDL levels in

premenopausal women are likely to protect them against atherosclerosis, and the difference may be casually related to Estradiol levels by Kalavathi et al.<sup>(18,19)</sup>

Testosterone had positive correlation with age( $r=0.164$ ) and lipid profile parameters except HDL-C( $r = -0.114$ ) had negative association in Table 3. Thus with increase of age, the Testosterone increases and it increases TC, LDL-C and decreases HDL-C in premenopausal women on comparison to the study in middle aged men.<sup>(20)</sup> Also this study accepts that higher Testosterone within the physiological range is less correlated with coronary artery atherosclerosis by Fredrick et al.<sup>(21)</sup>

DHEA is a precursor to both oestrogen and testosterone. In this study mean DHEA was ( $10.6 \pm 7.9$  ng/ml) in Group II (31-40yrs) was decreased compared to Group I (20-30yrs) of ( $12.04 \pm 6.9$ ng/ml) in Table 2. DHEA had negative association with age ( $r= -0.197$ ,  $p=0.03$ ) which is in accordance of fact that DHEA in women declines in a linear fashion with age.<sup>(21)</sup> DHEA decline in a linear fashion with age results in deficiency of androgens and estrogens in peripheral tissues proposed to be associated with age-related diseases of obesity and effect on cardiovascular risk factors and body fat.<sup>(12)</sup> The correlation analysis of DHEA with lipid profile was not significant. In this study DHEA showed a positive association with BMI ( $r=0.128$ ,  $p=0.16$ ) but a statistically significant negative association made out in De Pergola et al study.<sup>(21)</sup>

SHBG is a transport glycoprotein synthesized in the liver, normally stable in adults and in postmenopausal women. The mean SHBG was  $170.37 \pm 58.07$  in Group I and  $180.7 \pm 77.6$  in Group II with range of 52.5-209nmol/l observed to be increased than normal reference value. In Table-3 SHBG was negatively associated with BMI ( $r=-0.186$ ,  $p=0.04$ ).<sup>(22)</sup> Also shows there was no significant association with HDL-C ( $r=-0.03$ ) or TGL ( $r=-0.045$ ), whereas positive associations with HDL-C concentrations and negative associations with LDL-C, oxLDL-C, TGL, and ApoB levels present in Mary Franet al study.<sup>(17)</sup> The positive association of SHBG with TC ( $r=0.105$ ) was similar to Bell et al study.<sup>(23)</sup>

AIP reflect the true relationship between protective and atherogenic lipoprotein and is associated with the size of pre- and anti- atherogenic lipoprotein particle. AIP value less than 0.1 is associated with low risk, the values 0.1-0.24 intermediate risk and  $>0.24$  are associated with and increased risks of CVD.<sup>(13)</sup> The Mean AIP ( $0.28 \pm 0.05$ ) was increased depicting the women are at dyslipidemic risk. Testosterone showed positive association with TC( $r=0.262$ ), TG ( $r=0.208$ ), LDL-C ( $r=0.392$ ), AIP( $r=0.452$ ) and negative with HDL-C( $r=-0.114$ ) although statistically not significant considered to be a cardiovascular risk. Hypertriglyceridemia was observed in 36/120 (30%) women with a significant increase in mean Triglyceride

value  $153.03 \pm 37.3$  mg/dl in Group II (31-40yrs) ( $t=2.33$ ,  $p=0.02$ ). Androgens activate the expression of  $\beta$ -adrenergic receptors, Proteinkinase A and hormone sensitive lipase. As a result Testosterone stimulates lipolysis and thereby reduces fat storage in adipocytes. Androgens elicit an anti-adipogenic effect in preadipocytes in vitro, whereas estrogens behave as proadipogenic hormones.<sup>(21)</sup>

This study analysis shows definite dyslipidemia related cardiovascular risk among adult women exists even if their BMI is  $<23\text{kg/m}^2$  and estrogen levels within the normal physiological range. Mild androgenemia can lead to dyslipidemia.

## Conclusion

The adult women are definitely at risk of dyslipidemia, obesity and cardiovascular diseases as the across the age advances to menopause transition. Mild Testosterone increased and DHEA decreased with age was not observed to be at statistical significant level. SHBG is negatively associated with body mass index and positively association with Total cholesterol. Testosterone showed a positive association with TC, TG, LDL-C, Atherogenic index of plasma and negative association with HDL-C. Thus in reproductive and premenopausal period androgen levels influences dyslipidemia and can lead to cardiovascular risk. We recommend base-line androgen status and frequent determinations of lipid profile in adult women between 30-40years of age to manage and control the cardiovascular disorders. Therefore to put forth the sex hormones as independent risk factor of CVD needs to be followed up in the study subjects.

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