

A comparative study of serum estrogen and lipid profile in pre-menopausal and post-menopausal women as atherosclerotic risk factors

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Abstract

Objectives: Menopause is the permanent cessation of menstruation; since cardiovascular disease is the leading cause of death among post-menopausal women, which may be due to lower level of estrogen. The present study was undertaken to evaluate serum estrogen and lipid profile status in post-menopausal women and compare with premenopausal women.

Materials and Methods: 50 post-menopausal women and 50 pre-menopausal women in the reproductive age group were selected in the study. We measured serum estrogen and serum lipid profile [total cholesterol (TC), triglyceride (TG), HDL-Cholesterol, LDL-Cholesterol, and VLDL-Cholesterol.] in both pre and post-menopausal women. The comparison and correlation of estrogen level with lipid profile was done using correlation test and P value less than 0.05 was considered significant. Serum estrogen and lipid profile determinations were done by competitive binding immunoassay and enzymatic methods respectively.

Results: This study showed statistically significant lower values of serum estrogen in post-menopausal women compared to pre-menopausal women. ($p < 0.001$). Significant higher values of serum TC, TG, LDL-C and VLDL-C levels and significant lower values of HDL-C in post-menopausal women compared to pre-menopausal women ($p < 0.001$).

Conclusion: Our study showed altered serum lipid profile in post-menopausal women, hence they are at an increased risk for cardiovascular diseases. Estrogen may be the protective factor in premenopausal women. Therefore screening for lipid profile and specific health education is needed for all postmenopausal women to prevent the emerging cardiovascular diseases.

Keywords: Atherosclerosis, Cardiovascular disease, Estrogen, Lipid profile, Pre-menopausal, Post-menopausal.

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Introduction

Menopause is defined by The WHO as 'the permanent cessation of menstruation as a result of the loss of ovarian activity'.⁽¹⁾ A woman today will live approximately one third of her life after menopause.⁽²⁾ Menopause develops due to low estrogen production by disturbed hormonal cycle of ovulation.⁽³⁾

At the 21st century, CVD accounts for nearly 50% of all deaths in the developed world and 25% in the developing world.⁽⁴⁾ By 2020 it is predicted that CVD will surpass infectious disease as the world's number one cause of death and disability. This will lead to nearly one in every three deaths due to CVD in developing countries.⁽⁵⁾

Studies have shown that women are having lesser risk of developing CVD compared to their male counterparts before menopause, but this advantage will not be there after menopause. Data from the Framingham Study showed that female CVD rates accelerate faster than those of males after the age of 45 years.⁽²⁾

After menopause, the low estrogen production from ovaries results in changes of glucose and insulin metabolism, body fat distribution, coagulation, fibrinolysis and dysfunction of vascular endothelium,⁽²⁾ also derangement of lipoprotein profile.⁽⁶⁾ Estrogen have many cardio-protective functions that changes the

vascular tone by increasing nitrous oxide production. Among all the risk factors for CVD in women, estrogen deficiency after menopause has major role. The estrogen replacement therapy in postmenopausal women has reduced the risk of CVD by 25–50% by changing the lipid status.⁽⁷⁾

The traditional CVD coronary risk factors cannot explain the occurrence of CVD in certain number of patients, which has led to the search of new risk factors. Several factors leads to CVD risk hence study on endogenous hormones may improve our prediction of CVD.⁽²⁾ Experiments conducted in 20th century showed cholesterol as the main risk factor for atherosclerosis. Then studies done on characterization of lipoprotein practices led to the concept of in sudation of lipids as a cause for atherosclerosis.⁽⁵⁾ And LDL implicated as main risk factor in the development of CVD.⁽²⁾

Our study aimed to establish differences in lipid status in post-menopausal women and in young menstruating women and to investigate the relationship between the menopausal status and related hormonal variation of plasma lipid profile by serum estrogen level in both groups. The estrogen replacement therapy in postmenopausal women has reduced the risk of CVD by 25–50% by improving the lipid status.⁽⁸⁾ This suggest that to reduce risk of CVD in older women,

prevention and intervention of dyslipidaemia should begin early.

Materials and Method

Study was conducted at Raichur Institute of Medical Sciences and Rajiv Gandhi Super Speciality Hospital and Research Centre, Raichur, from September 2014 to September 2015. Women attending outpatient department and those working as house keepers in college were included in the study. The study comprised total 100 women, which included 50 Post-menopausal women who attained menopause at least 1 year before with daily moderate working habits and without any disease and disorder as cases. And 50 healthy pre-menopausal women of reproductive age group having regular periodic menses, with daily moderate working habits and without any disease and disorder as controls. Women with CVD, Hypertension, DM, any systemic disorder, any neoplasia, or any other inflammatory disease, and those who are on exogenous hormones and on hypolipidemic drugs were excluded from the study. And also to minimize the effect of life style on lipid profile, smokers, alcoholics, sedentary women and trained athletes or sports women were also excluded from the study.

After 12 hour overnight fasting 6 ml of venous blood samples collected from both the groups and estimated serum total cholesterol (TC) and serum triglycerides (TG), HDL and serum estrogen level. TC level estimated by CHOD-PAP method,⁽⁹⁾ TG level by

GPO Trinder method,⁽¹⁰⁾ HDL level by phosphotungstic acid method end point.¹¹ And LDL, VLDL values were calculated by applying Friedwald's equation.⁽¹²⁾

VLDL = TG/5 and LDL = TC - (VLDL + HDL)

Serum estrogen level by Enzyme linked fluorescent assay (ELFA) technique in VIDAS.⁽¹³⁾

The Body Mass Index. BMI gives a measure of relative weight, adjusted for height. This allows comparisons both within and between populations. The BMI is calculated as follows: BMI = (weight in kg)/(height in meters)² WHO Classification of Adult Categories of BMI.^(14,15)

Classification	BMI (kg/m ²)	Risk of comorbidities
Underweight	< 18.5	Low
Normal range	18.5 – 24.9	Average
Overweight	25.0 – 29.9	Mildly increased
Obese	>30.0	

Statistical Analysis: The results of all profiles Total Cholesterol, Triglyceride, LDL, VLDL, HDL, BMI, serum estrogen of 100 samples were expressed as mean \pm SD. Data was analysed using Microsoft excel, SPSS 22 version. Pearson's correlation was applied to correlate between the parameters. P-value of less than 0.05 was considered significant. And P value less than 0.001 was considered highly significant.

Results

Table 1: Comparison of parameters in both pre and post-menopausal women

Serum Biochemical parameters	Normal values ⁽⁷⁾	Pre-menopausal Women n=50 (mean \pm SD)	Post-menopausal women n=50 (mean \pm SD)
ESTROGEN (pg/ml)	43-575 in pre-menopausal <58 in post- menopausal	174.52 \pm 11.9	32.26 \pm 5.67**
TC (mg/dl)	<200	162.38 \pm 42.66	222.86 \pm 17.84**
TG (mg/dl)	<160	97.26 \pm 16.05	186.88 \pm 13.41**
LDL (mg/dl)	<100	90.79 \pm 48.26	154.8 \pm 20.21**
VLDL (mg/dl)	<32	19.45 \pm 3.21	37.3 \pm 2.68**
HDL (mg/dl)	>40	52.14 \pm 10.84	30.76 \pm 4.86**
BMI (Kg/m ²)	18.5-24.9	23.82 \pm 3.73	25.01 \pm 1.71*

* Significant p<0.05 ** Highly significant p< 0.001

Showing- Serum Estrogen level is high among premenopausal women than in post-menopausal women and Serum TC, TG, LDL and VLDL, BMI are raised among post-menopausal women compared to pre-menopausal women and the serum HDL values are decreased in post-menopausal women than pre-menopausal women and all the differences are statistically highly significant.

Correlative Study

Table 2: Pearson's correlation of serum Estrogen with lipid profile and BMI in pre-menopausal women

Estrogen	BMI	TC	TG	VLDL	LDL	HDL
Pearson's correlation	- 0.04	- 0.18	- 0.03	- 0.03	- 0.16	0.00
P-value	0.37	0.09	0.40	0.40	0.12	0.49
Significance	NS	S	NS	NS	NS	NS

Showing- Negative correlation of Estrogen with LDL and no correlation of Estrogen with HDL in pre-menopausal women

Table 3: Pearson's correlation of Estrogen with lipid profile and BMI in post-menopausal women

Estrogen	BMI	TC	TG	VLDL	LDL	HDL
Pearson's correlation	- 0.60	- 0.27	- 0.43	- 0.25	- 0.31	+ 0.43
P-value	0.000	0.02	0.001	0.04	0.01	0.001
Significance	S	S	HS	S	S	HS

Showing- Negative correlation of Estrogen with LDL and positive correlation of Estrogen with HDL in post-menopausal women

Discussion

CVD is a polyfactorial; dyslipidaemia is a major risk factor for atherosclerosis. Our study aimed to establish differences in lipid and hormonal status in post and pre-menopausal women and to investigate the comparison and correlation between the menopausal status and related hormonal variation with plasma lipid profile.

In Framingham cohort study, 2873 women were followed up for 24 years. Gordon T et al showed an increase in CVD incidence in postmenopausal women more than double compared to pre-menopausal women.⁽¹⁶⁾

In our study the postmenopausal women are having higher BMI compared to premenopausal women and p value $p < 0.05$. Srinivas Reddy Kilim et al⁽²⁾ found increased BMI in post-menopausal women but not significantly. Women have 3 times higher risk of CVD if BMI is 29 or higher.⁽¹⁷⁾ In our study BMI is negatively correlated with serum estrogen in both pre and post-menopausal women indicating that BMI directly related to CVD risk. Among postmenopausal women excess of fat accumulation is responsible for increased weight of the body.⁽¹⁸⁾ This excess of fat mass leads to excess of fatty acid release into circulation, which provides substrate for hepatic TG and lipoproteins rich in TG.⁽¹⁹⁾ Thus weight gain induces an increase in the plasma TG level.⁽¹⁷⁾

According to our study the Total Cholesterol, TG, LDL, VLDL values are increased among postmenopausal women compared to premenopausal women and difference is statistically highly significant ($p < 0.001$). And pre-menopausal women are having high HDL level than postmenopausal women and difference is statistically highly significant ($p < 0.001$). Our study showed significantly high level of serum estrogen in pre-menopausal women than post-

menopausal women with p-value < 0.001 . This finding is in accordance with study done by Srinivas Reddy Kilim et al⁽²⁾ and Swarnalatha et al.⁽²⁰⁾

TC is an independent risk factor for CVD. Razay Y et al⁽²¹⁾ showed that, 1%, increase in TC is associated with atleast 2% increase in the incidence of CVD. And he also showed that the postmenopausal women have higher TG value by 31% when compared to premenopausal women. He explained, as the plasma estrogen and progesterone level decreases in postmenopausal women, the lipoprotein lipase activity increases, this causes release of TG from adipocyte.⁽²²⁾ Hence plasma TG level increases after menopause.

Serum Estrogen is a regulator of lipoprotein lipase LPL. LPL catalyses the hydrolysis of VLDL to IDL and later to LDL. Estrogen also stimulates the synthesis of LDL receptors and ultimately reduces the plasma LDL level and in turn decreases the CVD risk in premenopausal women. After menopause due to low estrogen level leads to increased plasma LPL activity and also leads to decreased number of LDL receptors⁽²³⁾ which results in an increase in the plasma LDL level. Metabolic studies suggest that hormone replacement therapy produces reduction in LDL levels due to enhanced hepatic lipoprotein uptake, increased utilisation of hepatic cholesterol to bile acids, and increased expression of LDL receptors.⁽²⁴⁾

Postmenopausal women have low estrogen level, hence they have highest activity of hepatic lipase which enhances the uptake and catabolism of HDL thus decreases plasma HDL concentration.⁽⁷⁾ Cholesterol esterification is higher in plasma HDL in postmenopausal women as they have higher LCAT activity.⁽²⁵⁾ For every 10 mg/dl increase in HDL, there is a corresponding 50% decrease in CVD risk.⁽²⁶⁾ Studies done by Sultan N and others showed that postmenopausal women have increased risk for CVD

due to low estrogen level and low plasma HDL level after menopause. So HDL was an independent and better predictor of CVD risk in women.⁽²⁷⁾ The alteration in the lipid profile in women begins during the perimenopausal state. Within first 6 months of menopause, TC increased by 6%, TG by 11%, LDL by 10% and after 2 years of menopause HDL decreased by 6%.⁽²⁸⁾

Estrogen alters serum lipid concentration, blood coagulation, fibrinolytic activity, antioxidant systems and the production of NO and prostaglandins, which influence the development of vascular disease.⁽²⁹⁾ Estrogen decreases clotting factor VII, VIII, plasma fibrinogen and activity of plasminogen activator inhibitor, and also it increases anticoagulant proteins, antithrombin III and protein S. Estrogen inhibits platelet aggregation and reduce the reactivity of monocytes, thus estrogen has overall potential for fibrinolysis. Also estrogen reduces the entry of esterified cholesterol into the vessel wall and prevents the oxidative conversion of LDL in coronary arteries and also it inhibits endothelial and smooth muscle cell hyperplasia, collagen and elastin deposition of coronary arteries thus estrogen prevents atherosclerosis formation.⁽²⁸⁾

Rapid non genomic effects of estrogen is by directly activating estrogen receptor which regulate endothelial NOS through a tyrosine kinase pathway to produce NO which relaxes vascular smooth muscle and produce vasodilatation.^(5,29) Long term genomic effect of estrogen on the vasculature is by increasing the expression of genes for vasodilatory enzymes such as prostacyclin synthase and NOS.⁽²⁹⁾

The study conducted by Kalavathi et al concluded that, the premenopausal women have higher estrogen and HDL level and lower LDL level compared to postmenopausal women of same age group. These findings were likely to protect them against atherosclerosis and the difference was causally related to estrogen levels.⁽³⁰⁾

Our study has shown positive correlation of estrogen with HDL and negative correlation with LDL, VLDL, TC, TG. Study done by Srinivas Reddy Kilim⁽²⁾ showed positive correlation between estrogen with HDL and negative correlation between estrogen with LDL. Correlation of estrogen with lipid profile in study done by Swarnalatha et al⁽²⁰⁾ showed positive correlation with HDL and negative correlation with LDL, VLDL, TC, TG. Connor EB et al reviewed that, oral estrogen administration showed a 50% reduction in risk of coronary events in postmenopausal women.⁽³¹⁾

Conclusion

According to our study menopause is associated with increase in the CVD risk factors such as TC, TG, LDL, VLDL, BMI levels and decrease in HDL levels. The possible underlying mechanism for dyslipidaemia in post-menopausal women could be decreased estrogen level. Our study showed serum estrogen is negatively

correlated with TC, TG, LDL, VLDL, and BMI and positively correlated with HDL in both pre and post-menopausal women.

In order to modify risks of CVD in older women, intervention with regard to dyslipidaemia should begin in peri-menopausal period –like Periodic specific health education programmes, periodic screening tests, exercise, yoga, healthy diet habits etc.

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