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Original Research

Differential Evaluation Of Lipid Profile Parameter In Postmenopausal Women Of Different Ages

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ABSTRACT:

Objectives: Menopause induces the loss of ovarian hormones both estrogen and progesterone due to follicular depletion. Estrogen hormone regulates metabolism at cellular level directly by mRNA production for specific protein including proteins for lipid metabolism such as lipoprotein lipase (LPL) and hormone sensitive lipase (HSL) in adipose tissue. 17-beta-estradiol regulates the rate of synthesis of structural apolipoproteins for VLDL and HDL in liver. The lack of 17-beta-estradiol leads likely to various lipid metabolism disorders in women after menopause. The study aims at comparing the changes of serum lipid levels in control, premenopausal women with that of postmenopausal counterparts. **Method:** A total of 100healthy subjects {(75 of postmenopausal women, 12 were perimenopausal and 13 were control (whose menses cycle on time)} between ages from 24 to 75 years, were selected for the study. They were nonsmoker, non-addicted and non-user of any hormonal contraceptive. The lipid profile was assessed using standard methodologies. Statistical analysis were performed using the GraphPad Prism9 stat® Software. Analysis of data was done using one-way ANOVA employing Tukey's test. After analysis, $p<0.05$ was found to be statistically significant. **Results:** The sample for biochemical parametersviz Total Cholesterol, Triglyceride, HDL-Cholesterol, LDL-Cholesterol, VLDL-Cholesterol and Serum Estrogen levels of Controls, Perimenopausal and Postmenopausal women were tested and analysed. Mean value of serum estrogen in postmenopausal women was (22.8±1.0) mg/dl, in perimenopausal women was (95.0±19.0)mg/dl and in controls was (65.9±10.9) mg/dl. The average value of serum cholesterol levels of post-menopausal women was (207.8±6.2) mg/dl in perimenopausal women (190.9±8.1) mg/dl and in controls (168.3±5.4) mg/dl. The average value of serum triglycerides level in postmenopausal women was (230.3±13.6) mg/dl, in perimenopausal women was (156.7±7.4) mg/dl and in control was (108.2±7.5) mg/dl.However, value of serum HDL level in post-menopausal (24.1±1.8) mg/dl, in perimenopausal women (47.4±2.0) mg/dl and in control (42.1±1.4) mg/dl.The value of serum LDL levels in postmenopausal women (140.2±7.0) mg/dl in perimenopausal women was (111.9±5.7) mg/dl and control women were (104.6±3.6) mg/dl.The mean value of serum VLDL level in post-menopausal women (46.1±2.7) mg/dl, in perimenopausal women was (31.3±1.5) mg/dl and in control was (21.6±1.5) mg/dl. **Conclusion:** The decline of estrogen levels in postmenopausal women raised all lipid profiles (except HDL), but prominent effect was seen in VLDL and TG levels. Menopause leads to changes in lipid profile by reducing HDL, and elevating total cholesterol (TC), and triglycerides (TG), thus increasing the risk for cardiovascular events.

Keywords: Menstruation, estrogen, lipid metabolism, menopause, dyslipidemia.

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INTRODUCTION

Menopauseis the transition from the reproductive to the non-reproductive stage of life in women and is characterized clinically by permanent cessation of menstruation and biologically by loss of ovarian function. Menopause occurs around a mean age of 50years; virtually all women by the age of 55years or so willhave experienced menopause. The changes in birth andmortality rates over thelast century and, in particular, the profound decline in maternal mortality in developedcountries have resultedin an averagelife expectancy of women of about 75years; thus, most

women will be postmenopausal for one-third of their lifetime (Dosi*et al*., 2014).

Menopause is associated with different signs and symptoms, ranging from disturbances in lipid metabolism to psychological stress and sleep alterations (Auro*et al*.,2014).Early recognition of symptoms can help in the reduction of discomfort and fears among women. World Health Organization (WHO) has defined post-menopausal women as those women who have stopped menstrual bleeding one year ago or stopped having periods as a result of medical or surgical intervention (Hysterectomy/Oophorectomy) or both (Vaze*et al*., 2010). As indicated by the World Health Organization (WHO), natural menopause happens between the ages of 45 and 55 years for women around the world (Pallikadavath*et al*., 2016).

During the transition to menopause, changing hormone levels can affect your menstrual cycle and cause symptoms like hot flashes, vaginal problems and infections, problems in sleeping, pain during sex, memory problems, mood changes, depression and anxiety, changing feelings about sex, urinary problems, and irregular periods(Dalal*et al*.,2015).The main hormonal change in menopause includes low circulating levels of estrogen and marked an increase in luteinizing and follicle-stimulating hormone levels. There is considerable variation in the level of estrogen in postmenopausal women that occurs during the early postmenopausal years because of continued secretion of estradiol from the ovary and conversion of and rostenedione to estrone in fat tissue (Cui*et al*., 2016). Estrogen is mostly secreted by the ovarian follicles and the corpus lithium during pregnancy by the placenta.

Estrogen affects many parts of the body, including the blood vessels, heart, bone,breasts, uterus, urinary system, skin, and brain. Loss of estrogen is believed to be the cause of many of the symptoms associated with menopause. Prior to menopause, women are protected from myocardial infarction (MI) compared to age-matched men. The age of onset for the first myocardial infarction is ~10 years later for women compared to men. Furthermore, at any given age, women have one-third to one-half of the risk of cardiovascular disease relative to men (Iorga*et al*., 2017). Estrogen signaling pathways have pleotropic effects on many tissues and pathways that govern lipid and lipoprotein metabolism, but our understanding of these effects is complicated in that there are numerous endogenous estrogens and they differ between species (Palmisano*et al*., 2017). 17b estradiol is the predominant endogenous estrogen in humans. It is made by the ovaries and circulates in plasma associated with sex-hormone binding globulin. Estrogens are lipophilic steroid hormones that are thought to passively diffuse through the plasma membrane into the cytoplasm and nucleus where they bind the steroid nuclear hormone receptors, ERa and ERb (Fuentes*et al*., 2019). Unliganded ERa and ERb are kept inactive by association to Heat Shock Protein 90 (Hsp90) complexes. Binding of estrogens to ERa or ERb promotes dissociation from Hsp90, dimerization, and translocation into the nucleus to activate gene transcription (Fuentes*et al*., 2019).

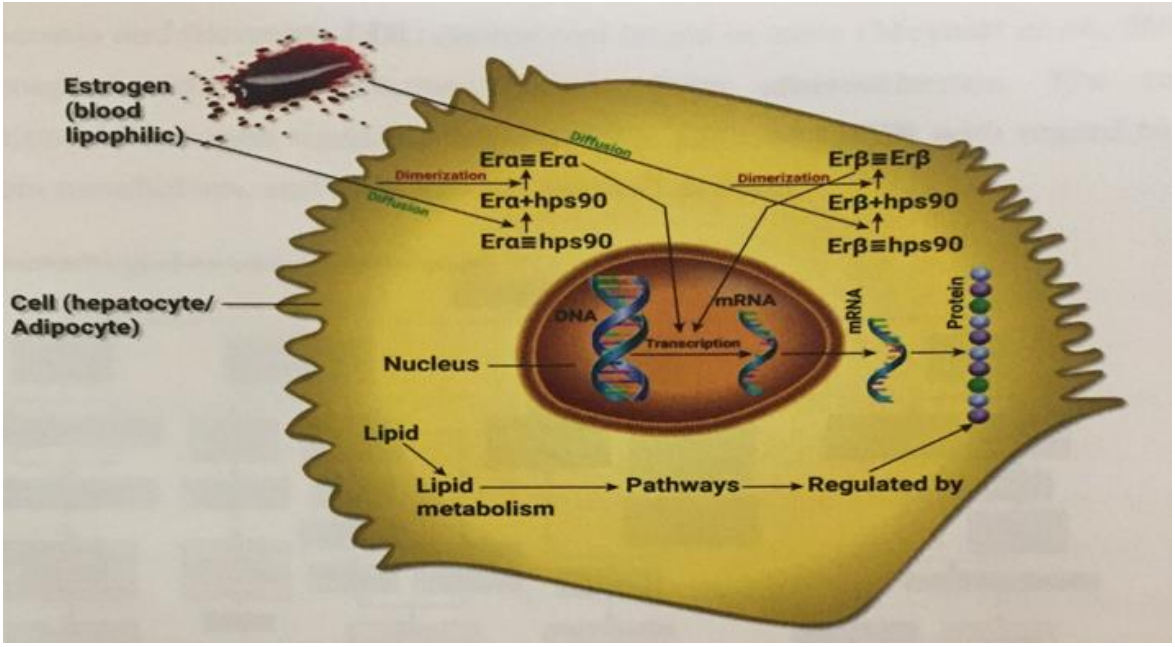

Figure 1: Modulation of lipid metabolism by estrogen and its analogues

The liver is an important site where fatty acids, TG, and cholesterol metabolism are coordinated to meet metabolic needs in normal physiology The primary movement of lipids between tissues occurs either as free fatty acids released by adipose tissue or in the form of lipoprotein carriers made primarily by the

liver and gut (chylomicrons and very-low-density lipoprotein (VLDL) for triglyceride (TG), and low-density lipoprotein (LDL) and high-density lipoprotein (HDL) for cholesterol) (Alves-Bezerra et al., 2017). Dyslipidemia, especially hypercholesterolemia, is the major risk factor of CVD. To contribute to the better understanding of lipid profile status in postmenopausal women, the present study was conducted to estimate the serum levels of total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-C),low-density lipoprotein cholesterol(LDL-C), very low-density lipoprotein cholesterol (VLDL-C), and atherogenic index and compare it with premenopausal women. Estrogen exerts cardio protective action by maintaining a high level of HDL-C and lowering the LDL-C and triglycerides level. Therefore, symptoms and diseases which are associated with estrogen deficiency are of increasing importance to women's health and so they are more proneto cardiovascular diseases after menopause (Couder*et al*., 1999).

Altered serum lipid profile is associated with menopause and thus it is a major determining factor predisposing to cardiovascular diseases. It makes the screening of postmenopausal women for abnormal lipid profile mandatory. Significance of serumoestradiol level and its correlation with lipid profile is to be investigated so that postmenopausal women can undergo further evaluation and earlier management (Saha et al., 2013).

MATERIAL AND METHODS

In this study, a total of 100 samples were taken out of which 13 women served as control who have regular mensuration cycle and belong to age below 45 years, other 75 were postmenopausal women with age group from 40 to 80 years and remaining 12 women served as perimenopausal women having irregular mensuration cycle. They were nonsmoker, non-addicted and non-user of any hormonal contraceptive.Blood sample were collected from Arora Hospital, and Bhakna Lab Diagnostic Centre Amritsar and tests were performed in Khalsa Diagnostic Lab Amritsar. The quantitative data was collected and applied accordingly. The serum total cholesterol (TC), high density lipoproteins (HDL-C), and triglyceride (TG) were estimated on semi-automated biochemistry analyzer (Erba Chem-5 Plus V2) using respective kits. Serum estrogen levels were determined by CLIA using SnibeMaglumi 600 analyzer.

RESULTS

To determine the values of lipid parameters in normal or high risk category, NCEP ATPIII criterion (https://www.nhlbi.nih.gov/files/docs/guidelines/atglance.pdf) was used. The quantitative variables were displayed as mean±standard error and percentage. The statistical difference for lipoproteins at 5% significance level was assessed by applying single sample "t" test.

1. Correlation between age, Weight and BMI of Controls, Perimenopausaland Postmenopausal women.

The average value of BMI inpostmenopausal women was (29.5±0.7)kg/m^2, in perimenopausal women (26.8±1.1)kg/m^{2}and in the control (27.5±1.6)kg/m^2. The mean value of BMI of postmenopausal women was higher as compared to perimenopausal women and control (Table-1)**.**

Table 1: Data of Age, Weight and BMI of Controls, Perimenopausal and Postmenopausal women.

S. No.	Subjects	Age (years)	Weight (kgs)	BMI (kg/m^2)
1	Controls	33.4±1.3	69±3.9	27.5±1.6
2	Perimenopausal women	44±0.8	64.8±3.2	26.8±1.1
3	Postmenopausal women	57.6±1.0	67.5±1.4	29.5±0.7

(Data representedasMean±SE)

2. Correlation of Total Cholesterol, Triglyceride, HDL-Cholesterol, LDL-Cholesterol, and VLDL-Cholesterol with Serum Estrogen levels of Controls, Perimenopausal and Postmenopausal women.

The mean value of serum estrogen in postmenopausal women was higher (22.8±1.0) mg/dl, as compared to perimenopausal women (95.0±19.0)mg/dl and controls (65.9±10.9) mg/dl. The value of serum cholesterol levels of post-menopausal women was significantly higher (207.8±6.2) mg/dl in comparison withperimenopausal women (190.9±8.1) mg/dl and controls (168.3±5.4) mg/dl. However, the serum triglycerides levels in postmenopausal women wereincreased (230.3±13.6) mg/dl, as compared to perimenopausal women (156.7±7.4) mg/dl and control (108.2±7.5) mg/dl. Serum HDL levels were lower in post-menopausal (24.1±1.8) mg/dl, in contrast to perimenopausal women (47.4±2.0) mg/dl and control (42.1±1.4) mg/dl. The serum LDL levels showed that postmenopausal women (140.2±7.0) mg/dl have an increased value as compared to perimenopausal women (111.9±5.7) mg/dl and controls (104.6±3.6) mg/dl. Whereas, serum VLDL levels calculated by friedewald's equation were higher in post-menopausal women (46.1±2.7) mg/dl, perimenopausal women was (31.3±1.5) mg/dl and in control was (21.6±1.5) mg/dl (Table-2).

Table 2: Data of biochemical parameters Total Cholesterol, Triglyceride, HDL-Cholesterol, LDL-Cholesterol, VLDL-Cholesterol and Serum Estrogen levels of Controls, Perimenopausaland Postmenopausal women.

S. No.	Subject	Serum Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Serum Estrogen (pg/ml)
1	Control group	168.3±5.4	108.2±7.5	42.1±1.4	104.6±3.6	21.6±1.5	65.9±10.9
2	Perimenopausal women	190.9±8.1	156.7±7.4	47.4±2.0	111.9±5.7	31.3±1.5	95.0±19.0
3	Postmenopausal women	207.8±6.2	230.3±13.6	24.1±1.8	140.2±7.0	46.1±2.7	22.8±1.0

(Data representedasMean±SE)

3. Differential evaluation of Levels of Serum Cholesterol, Triglyceride, HDL-Cholesterol, LDL Cholesterol and VLDL in postmenopausal women in different Estrogen ranges.

Estrogen levels of all subjects were arranged in three range groups- range-I (4-18pg/ml), range-II (18.1-32.2pg/ml) and range-III (>32.2pg/ml). The serum estrogen levels of all the 75 postmenopausal were analyzed. The result presented in (Table 3) indicates that out of a total of 75 postmenopausal women, 34% postmenopausal women (25) have estrogen levels in range-I, 53% postmenopausal women (40) have estrogen levels in range-II, 13% postmenopausal women (10) have estrogen level in range-III (**Fig. 2)**. The present study showed that maximum postmenopausal women have an estrogen level of 18.1-32 (Table 3).

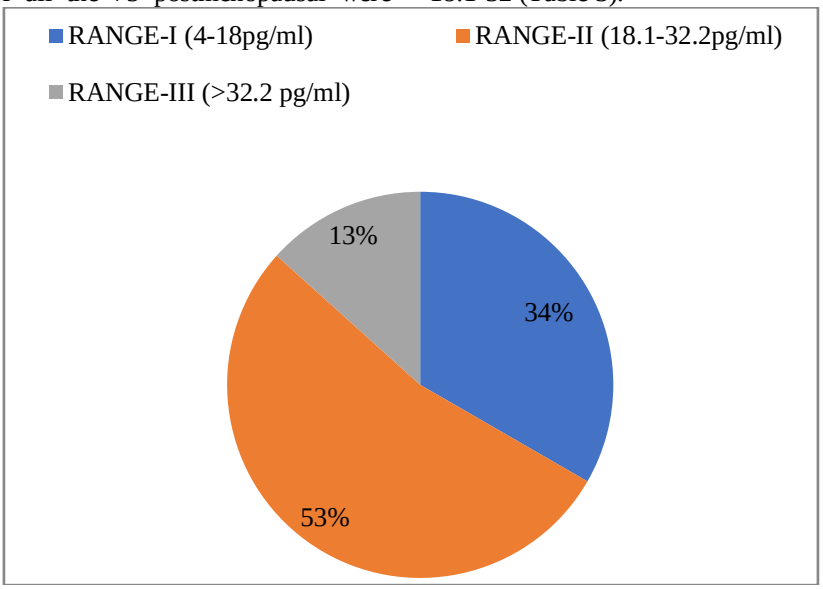

Figure 2:Showing Population %age of postmenopausal women with different Estrogen ranges.

Table 3: - Data of postmenopausal women in different Estrogen ranges

S.No.	Estrogen levels (pg/ml)	No. of postmenopausal women (75)	Percentage of postmenopausal women
Range I	4 – 18 (13.6)	25	34%
Range II	18.1- 32.2 (24.7)	40	53%
Range III	>32.2 (37.9)	10	13%

4. Correlation of different estrogen ranges with lipid profile at the high-risk level.

The percentage of different lipid parameters associated with the estrogen range-I illustrate that

24% of women have cholesterol, 56% women have TG, 12% women have HDL, 16% women have LDL, and 60% women have VLDL at high-risk levels. The percentage of different lipid parameters showed in the figure associated with estrogen range-II illustrate that 20% of women have cholesterol, 43% women have TG, 5% women have HDL, 25% women have LDL, and 50% women have VLDL at high-risk levels. The percentage of different lipid parameters showed in the figure associated with estrogen range-III illustrate that 20% of women have cholesterol, 20% women have TG, 0% women have HDL, 0% women have LDL, and 30% women have VLDL at high-risk levels (Table 4).

Table 4: Percentage data of Serum Cholesterol, TG, HDL, LDL, VLDL of postmenopausal women of different estrogen ranges.

Percentage (%) of no. of subjects															
Subject	**Cholesterol**			**Triglyceride**			**HDL**			**LDL**			**VLDL**		
	Normal	**Borderline**	**High risk**	**Normal**	**Borderline**	**High risk**	**Normal**	**Borderline**	**High risk**	**Normal**	**Borderline**	**High risk**	**Normal**	**Borderline**	**High risk**
Estrogen range-I (4-18pg/ml)	52	24	24	8	36	56	28	60	12	48	36	16	8	32	60
Estrogen range-I (18.1-32.2 pg/ml)	50	30	20	15	42	43	35	60	5	47	28	25	15	35	50
Estrogen range-I (>32.2 pg/ml)	10	70	20	40	40	20	0	100	0	10	90	0	40	30	30

5. Comparison of serum lipid levels and serum estrogen level between two groups based on duration of menopause in postmenopausal.

Mean value of serum estrogen in group-I (>5 years) and group-II (<5 years) of postmenopausal women was 26.7 and 20.8 respectively. The analysis of mean value of lipid profile in the different group based on duration of menopause in postmenopausal shows that in group-I the mean of serum cholesterol, TG, HDL, LDL and VLDL was 218.7±12.4 mg/dl , 206.7±14.8, 18.7±2.7, 158.6±12.7, and 41.3±3 respectively. In group-II value of serum cholesterol, TG, HDL, LDL and VLDL were 204.2±7.3, 233.7±17.6, 25.8±2.3, 135.5±8.7 and 46.7±3.5 respectively. Comparison showed that with the increase of duration of menopause year, level of serum estrogen decreases.

Table 5. Data of serum lipid levels and serum estrogen level between two groups based on duration of menopause in postmenopausal.

Duration of menopause	Groups	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Serum Estrogen (pg/ml)
<5 years	Group I	218.7±12.4	206.7±14.8	18.7±2.7	158.6±12.7	41.3±3.0	26.7±2.2
>5 years	Group II	204.2±7.3	233.7±17.6	25.8±2.3	135.5±8.7	46.7±3.5	20.8±1.1

(Data representedasMean±SE)

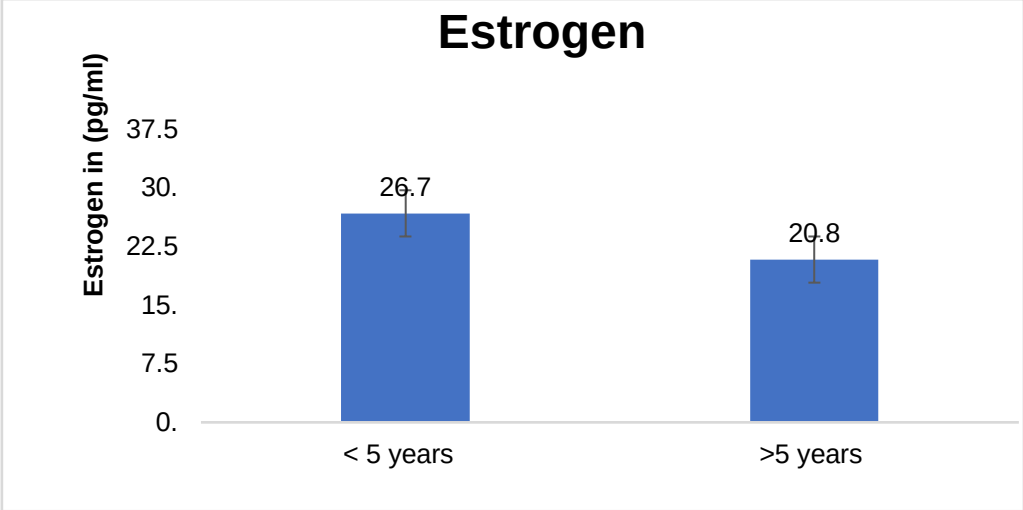

Figure 4: Showed mean value of serum estrogen in group -I (Duration of menopause<5 years) and group-II (Duration of menopause>5 years) of postmenopausal women.

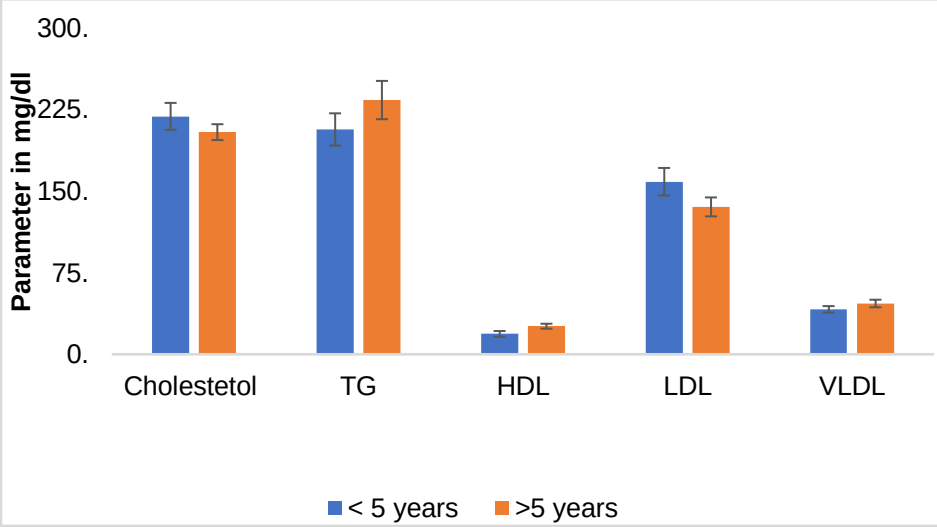

Figure 5:Showed data of mean value of lipid profile in the different group based on duration ofmenopause in postmenopausal.

DISCUSSION

The present study showed that the mean value of serum Estrogen was significantly higher ($p<0.05$) in postmenopausal women (22.8±1.0)mg/dl as compared to perimenopausal women (95.0±19.0)mg/dland control (65.9±10.9)mg/dl. After the onset of menopause, levels of estrogens slowly decrease because the number of ovarian follicles declines and the ovaries become less responsive to the two other hormones involved in reproduction—Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH). As the ovaries age, it releases fewer hormones, FSH and LH can no longer perform their usual functions to regulate estrogen, progesterone, and testosteronewhich leads to a natural decline of estrogen levels during menopause (Santoro *et al.*, 2007). Estrogen levels can increase the risk of obesity (Santoro *et al.*, 1996). The Body Mass Index (BMI) of postmenopausal women (29.5±0.7)kg/m^2 was higher as compared to perimenopausal (26.8±1.1)kg/m^2 women and control (27.5±1.6) kg/m^2. The serum cholesterol was significantly higher ($P<0.05$) in postmenopausal women (207.8±6.2)mg/dl than the perimenopausal women (190.9±8.1)mg/dl and control (168.3±5.4)mg/dl. The reason behind this could be that estrogen has a major beneficial effect on total cholesterol metabolism. Estrogen is responsible for balancing the cholesterol level. It hastens the turnover of cholesterol but when the Estrogen level decreases it becomes unavailable for cholesterol catabolism due to which levels of total cholesterol become high (Ariadi*et al.*,2019).The mean value of serum triglycerides (TG) postmenopausal women (230.3±13.6)mg/dl was significantly higher ($p<0.05$) than the perimenopausal women (156.7±7.4)mg/dl and control (108.2±7.5)mg/dl. The mean value of VLDL in postmenopausal women (46.1±2.7)mg/dl was significantly higher ($p<0.05$) in postmenopausal women than the perimenopausal women (31.3±1.5)mg/dl and control (21.6±1.5)mg/dl. VLDL

and TG are positively correlated with each other. Moreover, estrogen also contributed to the regulation of lipoprotein lipase, and lipoprotein lipase is responsible for hydrolyzing TG to chylomicrons and VLDL; Therefore, estrogen decrease during menopause could cause dysregulation of lipoprotein lipase (Chatterjee*et al.*, 2010).The circulating fatty acids are carried to the liver where they are converted to triacylglycerol and cholesterol. Estrogen has a major effect on triglycerides levels by promoting the use of lipid as fuel and increase the oxidation of free fatty acids (Genevieve*et al*., 2010)**.** A reduction in plasma triglyceride concentrations was observed in postmenopausal women after estradiol treatment but not progesterone or testosterone.The levels of serum HDL was significantly lower ($p<0.05$) in postmenopausal women (24.1±1.8) mg/dl than the perimenopausal women (47.4±2.0) mg/dl and control (42.1±1.4) mg/dl. HDL is positivelycorrelated with Estrogen. The mean value of postmenopausal women (140.2±7.0) mg/dl was more than perimenopausal women (111.9±5.7)mg/dl and control (104.6±3.6)mg/dl. The LDL levels were calculated by the friedwald'sequation so when the increase in the ratio of triglyceride than the LDL levels was gradually increased.

CONCLUSION

From this study, we concluded that Postmenopausal women have significantly higher ($p<0.05$) serum estrogen as compared to perimenopausal women and control. Women in menopause have lower concentration of HDL ($p<0.05$) and high concentration of Cholesterol ($p<0.05$), TG ($p<0.05$), LDL ($p<0.05$) and VLDL ($p<0.05$) in relation to women with regular menstruation. The concentration of estrogen shows negative correlation with VLDL and triglycerides concentration in women in menopause, while the correlation with HDL concentration is positive. Maximum postmenopausal women (53%) have estrogen level in range 18.1-32.2pg/ml whereas 34% postmenopausal women have estrogen level in range 4-18pg/ml and only 13% postmenopausal women have estrogen level in range >32.2pg/ml. As the estrogen level increased, the no. of women at high risk of having HDL, LDL, Cholesterol, TG, VLDL decreased. The decline of estrogen levels in postmenopausal women raised all lipid profiles (except HDL), but prominent effect was seen in VLDL and TG levels. Adverse changes in lipid profile in postmenopausal women under study have an increased risk of having complications of cardiovascular disease in near future. Early and timely detection and primary prevention can avoid morbidity and mortality in this high-risk population. Estrogens therapy in these postmenopausal women may result in the improvement of lipidmetabolism.

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