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Serum Lipid Profile in Menopause and Corrective effect of Hormone Replacement Therapy A Study based on Bilaspur District Chhattisgarh

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ABSTRACT: Menopause, defined as the last menstrual period in a woman's life, undergoes many significant changes called "climacteric changes". These changes are mainly caused by a lack of estrogen due to ovarian failure. The climacteric changes includes vasomotor hot flashes, sweating, vaginitis and vaginal atrophy, dyspareunia and loss of libido, mental dysfunction-loss of concentration, sleep disturbances, headache, mood swings, depression and loss of energy, altered skin and hair, skin and hair loss. An abnormal, cardio-protective lipid profile due to decreased levels of sex hormones—estrogen and progesterone—contributes to a dramatic increase in the incidence of ischemic heart disease and coronary heart disease in postmenopausal women. Coronary heart disease (CHD) is ten times more common in men than in women up to age 45. Estrogen and, to a lesser extent, progesterone also significantly reduce plasma cholesterol levels, possibly due to the action of these female hormones on lipoproteins. Plasma cholesterol levels are lower in women aged 20-45 than in men of the same age. If both ovaries are removed before the age of menopause, this is often followed by elevated plasma cholesterol and a greatly increased incidence of heart disease. The resulting dyslipidemia is one of the main causes of heart disease, especially ischemic heart disease in post-menopuased women. This was a Community based Case-control study on post-menopuased women including analysis of lipid profile and effect of HRT on serum lipid profile. Menopause was observed as a significant etio-pathological cause of dyslipidemia in studied post-menopaused subjects and positive corrective effect of HRT was seen on their lipid profile.

Keywords: Menopause, Dyslipidemia, Hormone Replacement Therapy, Cardiovascular Disease, Cholesterol, Lipid Profile.

INTRODUCTION

Cardio–vascular diseases represent an enormous medical, social and economic burden to the Indian public. CVD are the leading causes of social insecurity and disability now. Cardiovascular diseases, principally ischemic heart disease (IHD), are the most important cause of death and disability in the majority of low and lower-middle-income countries (LLMICs). Menopause does not directly cause cardiovascular diseases, certain changes that occur in the body during menopause can impact heart health as concluded by the study of Anna Jones (2023); Phan & Toth (2014). Menopausal transition with its incidental hormonal changes is considered to contribute to the development of metabolic syndrome especially Cardiac diseases (Pandey *et al*., 2010). In these countries, IHD mortality rates are significantly greater in individuals of a low socioeconomic status (SES) (Gupta and Yusuf 2019). Urbanization and altered life styles are indicators of socio- economic development and lead to risk factors for cardio- vascular diseases (CVD) (Begom & Singh 1995). According to India 2020 in India nearly 2.4 million deaths are caused by CVD, small scale community based studies indicate the prevalence of CVD in adults ranging from 2 to 6 % in rural and 6 to 10 % in urban areas. The Health Sector Review of the World Bank projects the CVD mortality rates are doubled between 2005-2018 in India. The incidence of dyslipidemia increases over a woman's lifespan, with adverse changes around the time of menopause (Torosyan *et al*., 2022) According to study done by Jeonghee and Mijin (2021) only 27.3% of Korean menopausal women over age 40 with dyslipidemia were aware of the condition. Ischemic heart disease is now the greatest scourge of the human population, particularly in the India. (Khan *et al*., 2020). There is a dramatic increase in the number of the elderly in the developing world. Today, there are approximately 580 million elderly in the world, out of which around 355 million live in developing countries (Report by National Institute of Social Defense, 2002; Anagnostis *et al*., 2022). In light of the projected growth of the population of older adults over the next
several decades, the societal burden attributable to CVD will continue to rise. There is thus, an enormous opportunity to foster successful aging and to increase functional life years through expanded efforts aimed at CVD prevention (Yazdanyar & Newman 2009), So, the problems of elderly are important to consider now, as they are big section of the society. Health problem is

their major problem (77%), of which heart problems are the principal ones (27.8%). The numbers of elderly women are more than the elderly men, (Shenoy & Verneka 2015), so a precise health policy should be planned for cardiac health of geriatric women. increased in aging women seems to indicate that pre-menopaused endogenous estrogens exerts a protective influence on women. Barrett-Connor *et al*. (1999). A study done by Nie *et al*. (2022) stated that estrogen-based menopause hormone therapy (MHT) could influence lipid profile in postmenopausal women in a protective way (Whitehead, 2006). The lowering of low-density lipoprotein cholesterol (LDL-C) is the primary target of therapy in the primary and secondary prevention of cardiovascular events (Phan & Toth 2014). Menopausal Hormone replacements (MHT) plays a positive role in maintaining healthy lipid profile in postmenopausal women, meanwhile for women with hypertriglyceridemia, low doses or transdermal MHT or tibolone would be a safer choice. Also a study done by (Phan & Toth 2014); Nie *et al*. (2022). Showed that hormone replacement therapy prevents age related vascular damages. Thus, this study is designed to investigate the problem of dyslipidemia in aged Indian women and also to study the effect of Hormone replacement therapy on their lipid profile. Such study work will provide new avenues of approach for instituting appropriated aggressive measures to prevent the development of coronary heart disease, which has been an important cause of slowly rising morbidity and mortality in Indian females especially. In this study an attempt has been made to correlate the influence of various risk factors, especially increasing cardio-damaging lipoproteins levels after menopause with the occurrence of ischemic heart disease in post-menopaused women (Aygen *et al*., 1999).

MATERIAL AND METHODS

The study was conducted in two parts

Part A. It was a case-control study based on analysis of serum Lipid Profile of Pre and Post menopaused women.

Subjects. The following two groups of participants were selected for the study

A. Control Groups

Human female volunteers of premenopausal age (22–40 years) were recruited to serve as participants. The total number of controls was 66 women. None of the participants had a family history of heart-related problems. No one had uncontrolled hypertension. Ischemic heart disease and myocardial infarction were reported in only three of sixty controls. None of the women studied had ever received hormonal treatment. The mean age of the control group was 34 years; their average weight was 52 kg. They belonged to different economic status, from high income. Menopausal transition with its incidental hormonal changes is considered to contribute to the development of Metabolic syndrome according to a study based on western India (Pandey *et al*., (2010). Thus, as not a single study is done in central India on this aspect, so this study is designed to assess the condition on preliminary level in a small study group.

B. Experimental Groups

This group included postmenopausal women (period since last menopause – 3 to 6 years). They ranged in age from 32 to 60+. Their average age was 46 years. The average body weight was 56 kg. Twenty-one out of sixty-six were reported to have various types and severity of cardiac problems, mainly ischemic and myocardial infarction. Eight of the sixty-six had a family history of heart disease. The experimental group also consisted of women of different economic status, (high to low income groups), different religions, with different dietary habits.

Part B. Effect of Hormone Replacement was observed on post menopaused women, 17 women were contacted in Department of Gynaecology, Apollo Hospital, Bilaspur Dr Kalpana Dash was the incharge, under her supervision the HRT was given. Four subjects were given oral hormone replacement therapy; other thirteen were given injectable form of hormone therapy according to their will. Their pre therapy and post therapy serum lipid profile was analyzed and compared, also the comparison was also done between oral and injectable form of hormone therapy.

Hormone Replacement Therapy. Hormone Replacement Therapy (HRT) is a general term given to estrogen therapy for pre-, peri-, and post-menopausal women suffering from disturbances in estrogen metabolism. To prevent unopposed estrogen-related endometrial hyperplasia and malignancy, it can be combined with a progestogen for the last 10-13 days of the cycle, where the uterus is still present. It may have the disadvantage of inducing withdrawal bleeds at regular intervals. However, it is not a contraceptive.

Preparations. Estrogens used include-Ethinyloestradiol, Mestranol, Estradiol, Oestriol and conjugated estrogens were studied in this work. A recent addition, tibolone, combines estrogenic and progestogenic activity with weak androgenic properties was also included as per advice given by Dr Dash. It was given continuously to a woman with an intact uterus, without cyclic progestogens.

Duration of Treatment. As HRT can be given till the age of 60 years for up to 10 years post-menopausally. This rule was followed in the studied group.

METHODOLOGY

Materials used: (Chemicals). The following chemicals and biochemicals were purchased and utilized.

1. Chema Diagnostica Qualigens fine chemicals A division of Glaxo India Ltd.
2. Span diagnostic limited, Surat, India.

(A) Cholesterol Estimation Kit (one step method of Wybenga and Plleggi) (Catalog No. – 25924)

(B) HDL Estimation Kit (One step method of Wybenga and Plleggi) (Catalog No.–25924)

(c)Triglyceride Estimation Kit (Enzymatic colorimetric method GPO–PAP liquid stable single regent) (Catalog No.
77034 (6×250ml)).

Participants. The following two groups of participants were selected for the present study

A. Control Groups

Human female volunteers of premenopausal age (22–40 years) were recruited to serve as participants. The total number of controls was 66 women. None of the participants had a family history of heart-related problems. No one had uncontrolled hypertension. Ischemic heart disease and myocardial infarction were reported in only three of sixty controls. None of the women studied had ever received hormonal treatment. The mean age of the control group was 34 years; their average weight was 52 kg. They belonged to different economic status, from high income group (monthly income more than 20,000/- month) to low income group (2000/- month) and belonged to different religions, their dietary habits were very different.

B. Experimental Groups

This group included postmenopausal women (period since last menopause – 3 to 6 years). They ranged in age from 32 to 60+. Their average age was 46 years. The average body weight was 56 kg. Twenty-one out of sixty-six were reported to have various types and severity of cardiac problems, mainly ischemic and myocardial infarction. Eight of the sixty-six had a family history of heart disease. The experimental group also consisted of women of different economic status, (high to low income groups), different religions, and different dietary habits.

Optical Measurements. We have Biochemistry Auto analyzer star 100 in our Biochemistry Lab, Lipid Profile analysis was done there, all routine colorimetric estimations were performed on Spectro-colorimeter 103 and Spectro-photometer 106, and Colorimeter 114, (5-filters) (Systronics, India) also available in Biochemistry Lab. **Blood Collection.** Blood sample (2 ml) was collected from control and experimental groups. Blood samples are collected preferably before meals. All analyzes were performed on serum and not plasma, as EDTA interferes with lipid estimation, particularly high-density lipoprotein (HDL).

Apart from blood collection and estimation in the lab, four reports of lipid profile analysis was collected in collaboration with Akash Patho-Lab, Bilaspur and two reporta were collected from the Pathologist, Sardar Patel City Hospital, Bilaspur; A report was also collected from Bose Pathological Lab Bilaspur. A relaxation analysis was performed. Blood samples were stored at cold temperature (4ºC) before estimation, if necessary, but discarded after 8 hours. It was equilibrated at room temperature before estimation.

Data related to lipid profile of pre-menopausal controls (count-. 66), 05 data were given as pooled data. The rest of them were analyzed, but they were in general agreement, so the collected and analyzed data were taken as a whole. Similarly, 05 out of 66 sample data of lipid profile of postmenopausal subjects were collected. The rest of them were analyzed, but they were in general agreement, so the collected and analyzed data were representative of the whole. **Isolation of Serum.** The blood samples obtained were stored at room temperature and then centrifuged at 4° to 8° C for 6 to 8 min at 3500 rpm to remove serum from the blood.

Estimation of Total Cholesterol. Cholesterol in blood samples was determined by the one-step procedure of Wybenga and Pleggii (Catalog No.-25924). This process is based on the oxidation of cholesterol to cholesterol oxidase (CHO). It is oxidized again to cholest 4-N 3-.One and hydrogen peroxide. Hydrogen peroxide reacts in the presence of 4-amino antipyrine and 4-chlorophenol peroxide (POD) to form a pink quinoneamine dye. The intensity of the color produced is proportional to the concentration of cholesterol in the sample. Briefly the assay involves the following reactions

$$\text{Cholesterol Esterase} \xrightarrow{\text{CE}} \text{Cholesterol} + \text{Fatty Acid}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{Choleste-4-en-3 one} + H_2O_2$$

$$H_2O_2 + \text{4-AAP} + \text{4- Chlorophenol} \xrightarrow{\text{POD}} \text{Quinoneimine} + H_2O$$

(Coloured Dye)

Protocol- Reagent 1 - Cholesterol reagent; Reagent 2 - Standard Cholesterol.

Serum, 0.25 ml and cholesterol reagent were thoroughly mixed in a 5.00 ml test tube and then placed in a boiling water bath for 90 seconds. The tube was then cooled to room temperature under running tap water. The optical density (O.D) of the test sample was read using a spectrophotometer at 560 nm. Standard cholesterol solution was prepared using 0.25 ml of standard cholesterol solution mixed with 5.0 ml of cholesterol reagent and followed the same steps as applied to serum samples.

A blank solution was prepared by adding 5.00 ml of cholesterol reagent to the test tube and performing the following steps as above. The absorbance of cholesterol standards and serum samples was read at 560 nm against a blank. All reagents of the kit are stable at 2-8°C. Because reagent 1–cholesterol reagents are corrosive, oral pipetting was avoided.

Linearity. This assay was linear up to 600-mg/100 ml cholesterol value.

Estimation of High Density Lipoprotein (HDL) mg/100 ml. HDL in blood/serum samples was determined by the procedure of De Aloysio *et al.* (1999). The process is based on the principle of production of hydrogen peroxide, which ultimately gives a blue color. The optical density of the developed color is measured at 600 nm, which is proportional to the HDL in the test sample.

For the estimation of HDL mg (%) a diagnostic kit from Span Diagnostics Ltd. (Catalog No. 25924) was used

based on the one-step method of Wybenga and Pileggi. The principle behind this procedure is that the anti-human β Lipo-protein Ig in reagent A binds to lipoproteins other than HDL (LDL, VLDL and Chylomicrons). This immuno-complex blocks cholesterol other than HDL. When reagent B is added, only HDL cholesterol reacts with the enzymatic chain (CHE-CO). The hydrogen peroxide produced by the enzymatic reaction obtains a blue-colored complex upon oxidative condensation with F-DAOS and 4-APP in the presence of peroxidase, the absorption of which is read. At 600 nm, proportional to the HDL cholesterol concentration in the sample.

Protocol. For estimation, 0.3 ml of fresh/stored serum was used. First, serum test samples were mixed with 0.3 ml of precipitating reagent (Polyethylene Glycol 16%, Additives and Stabilizers). It is used for the precipitation of lipoproteins-LDL and VLDL. Both are mixed well and then kept at room temperature for about 10 minutes. After this, the solution was centrifuged at 2000 rpm for 15 min. 0.2 ml of clear supernatant was taken and 5.0 ml of cholesterol reagent was added to it. The contents were mixed well and then the tube was immediately placed in a boiling water bath for 90 seconds and immediately cooled to room temperature under running tap water. Optical density was read on a spectrophotometer at 600 nm. The same procedure was applied to prepare the standard solution. The color developed was stable for at least 10 minutes. It was kept away from strong light sources.

Linearity - This assay was linear up to 400 mg/100 ml levels.

Estimation of Triglyceride. (TG) mg/100 ml. Triglycerides in the blood/serum samples were determined by the procedure of Bucolo & David (1973). The procedure is based on the principal of production of red colored dye, Quinoneimine, which absorbs sharply at 510 nm. Briefly the assay comprises to the following reactions-

$$\text{1) Triglyceride} \xrightarrow[\text{Lipase (serum/microbial)}]{} \text{Glycerol + Fatty Acids}$$

$$\text{2) Glycerol +ATP} \xrightarrow[\text{Glycerol Kinase}]{} \text{Glycerol 3- phosphate + ADP}$$

$$\text{3) Glycerol-3-phosphate+} O_2 \xrightarrow[\text{Glycerol Phosphate Oxidase}]{} \text{Dihydroxy acetone phosphate+ } H_2O_2$$

$$\text{4) } H_2O_2\text{+4-AAP+4-chlorophenol} \xrightarrow[\text{Peroxidase}]{} \text{Quinoneimine+} H_2O$$
(Colored Dye)

For the estimation of triglyceride (mg/100 ml) a diagnostic kit from Chema diagnostics Ltd Glaxo– free of azide (Catalog No. 77034) (6×250 ml) was used. Triglycerides in samples are hydrolyzed to glycerol and free fatty acids (FFA) by microbial lipases. Glycerol is phosphorylated by adenosine 5-triphosphate (ATP) to glycerol-3-phosphate (G-3-P) in a reaction catalyzed by glycerol-kinase (GK). G-3-P is oxidized to dihydroxy-acetone phosphate (DAP).) in a reaction catalyzed by the enzyme glycerol phosphate oxidase (GOP). In this reaction hydrogen peroxide (H2O2) is produced in a concentration proportional to the level of triglyceride present in the sample. H_2O_2 reacts with 4-Amino-antipyrine (4-AAP)) and 4-chlorophenol, in a reaction catalyzed by peroxidase (POD). The result of this oxidative coupling is quinoneamine, a red dye. The absorbance of this dye in the solution is proportional to the concentration of triglycerides in the sample.

The reagents of the kit were already supplied in liquid form, ready to use. The kit was used for in vitro diagnosis.

Protocol. Serum, 0.02 ml was mixed with 2 ml reagent. Both were mixed well and incubate at 37° C for 5-8 min. The optical density was read at 510 nm in spectrophotometer. The same procedure was carried out for preparing standard solution. The absorbance of test and standard solutions were read at 510 nm against blank reagent.

Linearity. This assay was linear at least to 1000 mg/100 ml Triglyceride value.

Calculations. The following formula was used to determine the mg/100 ml value of the following

(A) Total Cholesterol

(Normal level -130-250 mg/100 ml in adults)

$$\text{Serum Cholesterol (mg/100 ml)} = \frac{\text{Optical density of Test (Ax)}}{\text{Optical density of Standard (As)}} \times 200$$

(B) HIGH DENSITY LIPO-PROTEIN (HDL)

(Normal level - 35-75-mg/100 ml for adult female)

$$\text{HDL (mg/100 ml)} = \frac{\text{Optical density of Test}}{\text{Optical density of standard}} \times 50$$

(C) ESTIMATION OF TRIGLYCERIDE (TG)-

(Normal level = 10-190 mg /100 ml for adult woman)

$$\text{TG (mg/100 ml)} = \frac{\text{Optical density of test}}{\text{Optical density of standard}} \times 200$$

(D) CALCULATION OF LOW-DENSITY LIPOPROTEIN (LDL mg /100 ML)

For this the **fw** formula was adopted = (Normal Range = 30-60 mg/100 ml)

(A) Triglyceride mg/100 ml = **x**

(B) X + HDL mg/100 ml = **y**

(C) Total cholesterol –**y** = **LDL** (mg /100 ml)

RESULT AND DISCUSSION

(A) Study 1: As can be appreciated from the above data, significant differences in various lipid components of both pre-menopausal and post-menopausal groups are noteworthy. The calculated t values are significant at both levels, indicating that lipid-profile changes have occurred in the postmenopausal state. Compared to the premenopausal group, the postmenopausal group showed a 48% increase in the cholesterol fraction, a 53% increase in the triglyceride fraction, a 51%

increase in the HDL fraction, and a 131% increase in the LDL fraction. These results show that when the lipid profiles of pre-menopausal and post-menopausal women were compared in all four parameters, serum total cholesterol showed significant differences between the two groups. A similar trend was observed in the triglyceride portion of the lipid spectrum, but both groups showed significant differences in their high-density lipoprotein portion and low-density lipoprotein portion. HDL levels observed in the post-menopausal group were significantly lower than those in the pre-menopausal group. It is clear from Table 3 that the fraction of LDL showed a reverse trend, being lower in the pre-menopausal group and comparatively higher in the post-menopausal group.

(B) Study 2: Deficiency of estrogen and progesterone resulted in disturbance of many physiological functions such as osteoporosis, body's Calcium loss, joint pain, rigidity of joints, dry and wrinkled skin, scalp hair loss, excessive sweating, hot flashes, mental instability and depression, increased myocardial infractions with reduced left-ventricular circulation, increased uterine and vaginal infections, increased possibilities of tumors and fibroids in uterus and ovaries, hirsutism, irregular menstrual bleeding, Hormone replacement therapy is usually suggested as remedy by endocrinologists. It includes oral, transdermal, or transvaginal administration of estrogen or progesterone or both together. When the uterus is removed, commonly only estrogen therapy is given, if uterus is intact combined hormone replacement therapy is suggested. In cases of intact uterus, the replacement of only estrogen results in hyperplasia of endometrial muscle culture, which can convert in uterine cancer also. In the present research seventeen such participants were found who were taking this therapy. All participants were taking hormone therapy from Dr. Kalpana Dash, Endocrinologist, Apollo Hospital (Bilaspur). Out of seventeen patients, most of them were taking conjugated estrogen (11 participants), two were taking combined hormone replacement therapy (Tibolone= oestriol + medroxy progesterone acetate) and four women were taking oral form of conjugated hormone: Premarin. They were taking HRT due to different causes as- Hirsutism, Post-menopausal osteoporosis, Dry skin and hair, and to slow down the aging process etc. The daily dose was 0.625 mg/day. The duration of the replacement course was 6 to 8 weeks. Their composition of lipid profile was taken preferably before the start of the therapy and after the completion of the course.

It is clear from the above data; significant differences in various lipid constituents of both oral and injectable hormone replacement therapy are noteworthy. The calculated t values are significant at both levels in the case of Cholesterol, LDL and HDL in the case of injectable form & in the case of LDL with oral forms.

The study showed a remarkable change after 6-8 weeks of HRT. The oral form of HRT reported a 31% decrease in cholesterol, a 111% increase in HDL, and a 64% decrease in LDL, while the injectable form of HRT resulted in a 39% decrease in cholesterol, a 107% increase in HDL, and a 70% increase in HDL. A % reduction in LDL fraction was noted. A significant decrease in serum cholesterol was observed after HRT. Cholesterol, with an initial level of 2.14 mg/ml, decreased to 1.35 mg/ml after therapy, a significant difference. This reduction was a remarkable 39% compared to pre-HRT conditions. Triglyceride fraction was found to be unaffected by hormone therapy. There was a significant difference in HDL by therapy.

These results are in trend with the study done by Gregersen *et al*. (2019); Anagnostis *et al*. (2022).

Table 1: The Composition of Lipid Profile of Pre-Menopaused Women.

Age Group	No. of Participants	Total Cholesterol	Triglyceride	HDL	LDL
25-28	9	1.12±0.33	0.82±0.24	0.51±0.10	0.45±0.14
29-32	14	1.23±0.12	0.84±0.23	0.52±0.07	0.54±0.13
33-36	10	1.32±0.14	1.16±0.34	0.49±0.08	0.60±0.10
37-40	14	1.18±0.18	0.75±0.35	0.52±0.09	0.52±0.12
41-44	13	1.35±0.25	1.30±0.28	0.49±0.10	0.53±0.13
45+	6	1.30±0.22	1.03±0.43	0.52±0.10	0.58±0.25

n = 66, (Values expressed as x $mg.ml^{-1}$ serum and are presented as Mean value ± Standard Deviation).

Table 2: The Composition of Lipid Profile of Post-Menopaused Women

Age group	No. of Participants	Total Cholesterol	Triglyceride	HDL	LDL
37-40	12	2.02±0.33	0.95±0.29	0.29±0.06	1.46±0.24
41-44	11	2.07±0.25	1.63±0.38	0.27±0.08	1.49±0.19
45-48	12	1.50±0.35	1.11±0.39	0.31±0.07	0.98±0.31
49-52	9	1.83±0.26	1.24±0.37	0.30±0.09	1.29±0.22
53-56	10	1.88±0.25	1.87±0.28	0.34±0.07	1.17±0.19
57-60	8	1.76±0.22	1.76±0.46	0.27±0.05	1.13±0.17
60+	4	1.89±0.17	1.91±0.42	0.28±0.02	1.23±0.16

n = 66, (Values expressed as x $mg.ml^{-1}$ serum and are presented as Mean value ± Standard Deviation)

Table 3: Mean SD & 't' values of lipid profile of pre -and post – menopaused women.

Factors of Lipid Profile	(mean ± Sd)		Change in percent-age value	(df = 130) t value
	Pre-Menopaused (n=66)	Post-Menopaused (n=66)		
Cholesterol(mg/ml)	1.25 (±0.08)	1.85 (±0.17)	□□□	17.63*,**
Triglyceride(mg/ml)	0.98 (±0.20)	1.49 (±0.36)	↑ 53%	18.68*,**
HDL (mg/ml)	0.59 (±0.18)	0.29 (±0.02)	↓ 51%	24.29,*,**
LDL (mg/ml)	0.54 (±0.16)	1.26 (±0.17)	↑ 131%	49.00,*,**

***** P<0.05 level; **P<0.01 level. SD Values showed in parenthesis.

Table 4: (Effect of hormone replacement on lipid profile).

A. Total Cholesterol profiling.

Age of each case	Before HRT	After HRT
29	2.27	1.37
33	2.40	1.48
34(oral)	1.97	1.45
36	2.00	1.48
40	2.20	1.44
42(oral)	2.10	1.33
45	2.02	1.49
47(oral)	1.88	1.36
49	1.86	1.33
49	2.09	1.24
50	2.26	1.39
53(oral)	2.04	1.39
55	2.41	1.30
55	1.97	1.27
56	2.36	1.35
56	2.27	1.32
56	2.34	1.04

n =17 (Values expressed as x $mg.ml^{-1}$ serum and are presented as Mean value ± Standard Deviation).

Table 4 B: HDL Profiling.

Age in each case	Before HRT	After HRT
29	0.27	0.58
33	0.30	0.63
34(oral)	0.26	0.64
36	0.31	0.59
40	0.26	0.53
42(oral)	0.32	0.58
45	0.30	0.55
47(oral)	0.27	0.58
49	0.30	0.63
49	0.26	0.64
50	0.31	0.60
53(oral)	0.28	0.54
55	0.32	0.58
55	0.30	0.55
56	0.27	0.58
56	0.30	0.64
56	0.26	0.65

n =17 (Values expressed as x $mg.ml^{-1}$ serum and are presented as Mean value ± Standard Deviation).

Table 4C: Triglyceride Profiling.

Age in each case	Before HRT	After HRT
29	2.00	1.99
33	1.70	1.68
34(oral)	1.10	1.08
36	0.97	0.97
40	1.34	1.32
42(oral)	1.86	1.84
45	1.63	1.62
47(oral)	1.72	1.71
49	1.26	1.27
49	1.17	1.19
50	0.99	1.01
53(oral)	1.08	1.06
55	1.92	1.89
55	1.57	1.54
56	1.63	1.61
56	1.47	1.44
56	1.02	1.00

n =17 (Values expressed as x $mg.ml^{-1}$ serum and are presented as Mean value ± Standard Deviation).

Table 4 D: LDL Profiling.

Age in each case	Before HRT	After HRT
29	1.60	0.39
33	1.76	0.51
34(oral)	1.49	0.59
36	1.49	0.69
40	1.67	0.64
42(oral)	1.41	0.38
45	1.39	0.62
47(oral)	1.27	0.44
49	1.31	0.45
49	1.60	0.36
50	1.52	0.59
53(oral)	1.54	0.64
55	1.71	0.34
55	1.36	0.42
56	1.76	0.45
56	1.68	0.40
56	1.88	0.19

n =17 (Values expressed as x $mg.ml^{-1}$ serum and are presented as Mean value ± Standard Deviation).

Table 5: Mean SD & 't' values of lipid profile in participants pre- and post-hormone replacement therapy.

Lipid Constituents	(Mean ± SD)		Change in percentage value	(df = 32) t value
	Pre HRT	Post HRT		
Cholesterol (mg/ml)	2.14 (±0.18)	1.36 (±0.10)	↑ 37%	5.13*,**
Triglyceride (mg/ml)	1.44 (±0.34)	1.43 (±0.33)	↑ 1%	0.10 NS
HDL (mg/ml)	0.29 (±0.02)	0.59 (±0.04)	↓ 103%	7.65*,**
LDL (mg/ml)	1.56 (±0.17)	0.48 (±0.13)	↑ 70%	1.09 NS

NS = Not Significant, * P<0.05 level, **P<0.01 level; [SD values showed in parenthesis]

As can be appreciated from the above data, significant differences in various lipid constituents of both before and after hormone replacement therapy are noteworthy. The calculated *t* values are significant in the case of Cholesterol and HDL.

Table 6: Mean, SD & 't' Values of Lipid Profile of Receivers of Oral/ Injectable form of HRT.

Lipid Constituents	(Mean ± SD)						t values	
	Oral (N=4)			Inject able (N=13)				
	Pre HRT	Post HRT	Change in % value	Pre HRT	Post HRT	Change in % value	Oral (df= 6)	Inject able (df = 24)
Cholesterol (mg/ml)	2.00±0.08	1.38±0.05	↑ 31%	2.19±0.17	1.35±0.12	↑ 39%	0.88 NS	4.69*,**
Triglyceride (mg/ml)	1.44± 0.34	1.4± 0.36	↑ 1%	1.44± 0.33	1.43±0.32	↑ 1%	0.03 NS	0.08 NS
HDL (mg/ml)	0.28±0.02	0.59±0.03	↓ 111%	0.29±0.02	0.60±0.04	↓ 103%	0.16 NS	6.61*,**
LDL (mg/ml)	1.43±0.10	0.51±0.11	↑ 64%	1.59±0.17	0.47±0.14	↑ 71%	2.09*,**	9.71*,**

NS = Not Significant, * P<0.05 level, **P<0.01 level

CONCLUSIONS

When the lipid profiles of premenopausal and postmenopausal women were compared, in all four parameters, serum total cholesterol showed significant differences between the two groups. The same pattern was seen with triglyceride levels, but both groups showed a marked difference in their high-density lipoprotein fraction and low-density lipoprotein fraction. HDL was found to be 0.29 mg/ml in the post-menopausal group, which was significantly lower than the HDL level found in the pre-menopausal group, which was 0.59 mg/ml. The LDL fraction showed the opposite trend, being lower in the pre-menopausal group (0.54mg/ml) and relatively higher in the postmenopausal group (1.26 mg/ml). A significant difference was found between the two groups, which was significant at both the 1% and 5% levels. Compared to the premenopausal group, the postmenopausal group showed a 48% increase in the cholesterol fraction, a 52% increase in the triglyceride fraction, a 51% increase in the HDL fraction, and a 131% increase in the LDL fraction. High levels of HDL is cardio-protective in pre-menopuased women, its a good cholesterol that cleans blood vessels, while LDL on the other hand causes narrowing of blood vessels and leads to cardiac ischemia and eventual arrest. This healthy trend is seen in pre-menopausal women with higher HDL and lower LDL and abnormal lipid profile with lower HDL and higher LDL in postmenopausal women is observed. In one study it was concluded that, this pattern of lipid profile composition can be dangerous because it has an inherent potential to cause heart attacks (Almenar *et al.*, 1997).

Total cholesterol increases in postmenopausal women, probably due to the lack of estrogen and progesterone in those women. (Torosyan *et al.*, 2022) similarly, changes in HDL showed a cardio-negative trend after menopause. A significant decrease in this good cholesterol has been reported after menopause. Triglyceride fraction was not significantly different between the two groups. The same observations were noted by the study done by Jeong & Kim (2022). All these changes in the profile after menopause makes women more prone to cardiovascular diseases, especially ischemic diseases. Our study showed that the presence of enough female hormones—estrogen and progesterone in the body of pre-menopausal women may have a protective effect against heart problems by influencing the lipid profile. This may be the reason that in the experimental group of 66 postmenopausal women, 21 (32%) reported some degree of cardiac problems, mainly cardiac ischemia, while in the same number of pre-menopausal group (66), only 3 women showed this trend. Out of 66 (4.54%) were reported to have mild cardiac disorder.

By HRT, our studies further support the findings that the estrogen plays a very important role in preventing cardiac problems, possibly due to preventive role of feminine hormones against dyslipidemias (Sreeniwas & Sinha 2020).

Participants who were taking oral hormones showed no significant difference after therapy. Only significant changes were observed in LDL fraction. Oral preparations are not preferred mostly, because they first pass the liver and intestines based metabolism and destroyed to some extent (*Liver Tox: Clinical and Research Information on Drug-Induced Liver Injury*. (2012). Some previous studies have shown that the oral form increases renin substrate production (particularly dangerous for hypertensive participants) and increases thromboembolic disease in people with a positive history (Whitehead, 2006).

Interestingly, the present study found the transdermal preparation more effective in improving serum lipid conditions. It significantly alters all serum lipid parameters except triglyceride in a cardio-friendly manner. Transdermal and sub-cutaneous implants may more closely reflect endogenous hormone activity. This finding matches with the work of Gregersen *et al.* (2019). Subcutaneous estrogen produces a plasma estrogen profile that mimics the ovulatory cycle. Thus the choice of estrogen depends on indication, risk, convenience and patient compliance. Our data provide evidence consistent with previous findings that HRT improves quality and duration of life in postmenopausal women. A lower incidence of stroke and myocardial infarction is also a great benefit. Altering the lipid profile in such a cardio-friendly manner showed that the overall effect of HRT was beneficial to the heart (Alwers *et al.*, 1999).

FUTURE SCOPE

As the incidences of cardiac problems are increasing tremendously in India with increasing numbers of geriatric females, thus post menopausal dyslipidemia and hormonal replacement therapy should be considered while studying/ planning for prevention of the occurrence of CVD among aged women.

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Conflict of Interest. None.

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