

COMPARATIVE ASSESSMENT OF LIPID PROFILE IN PRE-MENOPAUSAL AND MENOPAUSAL WOMEN IN NNEWI NIGERIA

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Abstract

The study was aimed at determining the effect of menopause on lipid profile. A total of 100 apparently healthy subjects who comprised 50 menopausal women aged 45 – 77 years and 50 pre-menopausal women between the aged between 20-52 years were recruited. Ethical approval was obtained from the Faculty of Health Science and Technology, Nnamdi Azikiwe University ethics committee and informed consent of each participant was obtained prior to recruitment. We estimated serum levels of total cholesterol using the enzymatic end point method as described by Roeschlau *et al.*, (1974), HDL was performed using the combination of phosphotungstate precipitation and enzymatic method as described by Burstein *et al.*, (1980), LDL by the combination of polyvinyl sulphate precipitation and enzymatic method of Assman *et al.*, (1984), VLDL was estimated using the method as described by Friedwald *et al.*, (1972) and triglycerides by the enzymatic method as described by Tietz (1990). The results shows that the mean levels of serum triglycerides (TG) and low density lipoprotein cholesterol (LDL cholesterol) showed a statistically

significant increase in menopausal women compared with the premenopausal subjects ($P < 0.05$) while there was no significant difference in the mean values of serum high density lipoprotein cholesterol (HDL cholesterol), very low density lipoprotein cholesterol (VLDL cholesterol) and total cholesterol ($P > 0.05$). There was a progressive increase in the mean levels of total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol with duration of menopause while the levels of HDL-cholesterol decreased with duration of menopause. There were no significant variations among the various age categories of the menopausal subjects and the levels of the lipid parameters studied ($p > 0.05$). The findings suggest that premenopausal women have less proatherogenic lipid profile than their menopausal counterparts.

Keywords: Menopause, Pre-menopausal, Lipid profile

Introduction

Menopause is the permanent cessation of menstruation at the end of reproductive life due to loss of ovarian follicular activity (Dutta, 2008). The effect of the hormonal changes associated with menopause on the serum lipid levels play important roles in most cardiac related disorders associated with menopause (Do *et al.*, 2000). Up to the age of 50 years, the prevalence of coronary artery disease (CAD) among women is lower than among men, but the incidence rises significantly after the menopause. The incidences of coronary heart disease have been observed to be increased in postmenopausal women until they become similar to the corresponding rates in men of similar age (Berg, 2004). Multiple risk factors have been identified as contributory to the development of CAD. Hypercholesterolemia is a key factor in the pathophysiology of atherosclerosis (Igweh *et al.*, 2005). After menopause, there is loss of ovarian function. This results in adverse changes in glucose and insulin metabolism, body fat distribution, coagulation, fibrinolysis, vascular endothelial dysfunction and also derangement of lipoprotein profile. Lack of estrogen is an essential factor in this mechanism (Bales, 2000; Samaan and Crawford, 1995).

Lipid profile or lipid panel is a panel of blood tests that serves as an initial broad medical screening tool for abnormalities in lipids, such as cholesterol and triglycerides. The results of this test can identify certain genetic diseases and can determine approximate risks for cardiovascular diseases, certain forms of pancreatitis and other diseases (Usono *et al.*, 2006). Lipid profile has been proven to be good indicators of whether someone is likely to have a heart attack or stroke, caused by blockage of blood vessels or hardening of the arteries (atherosclerosis). The lipid profile typically includes; total cholesterol (TC), high-density lipoprotein cholesterol (HDL-

C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG). Using these values, very low-density lipoprotein cholesterol (VLDL-C) can be calculated (Sidhu and Naugler, 2012).

Whether dyslipidemia leads to significant increase in the development of coronary artery disease (CAD) is still controversial, more so in our environment where little work has been done. The behaviour of lipoproteins during the menopausal transition and their relationship with the sex hormones and body fat distribution is still unclear (Berg, 2004). Thus, it is in view of the above findings that this research was designed to ascertain the risk of coronary heart diseases in menopausal women by evaluating the lipid profile status in pre-menopausal and post-menopausal women.

Materials and methods

Study site

This research was done in Nnewi metropolis, Nnewi North L.G.A, Anambra state, Nigeria.

Study population

The study population consisted of 100 apparently healthy subjects who comprised 50 menopausal women and 50 pre-menopausal women between the age limits of 45-77 and 20-52 respectively.

Inclusion criteria

The research study was concerned with postmenopausal women between the age limit of 45-77 and premenopausal women between the age limit of 20 to 52.

Exclusion criteria

The subjects having risk factors that may affect the lipid profile such as, diabetes mellitus (DM), neoplasia or other serious pathological disorders, those outside the age bracket and those on hormonal replacement therapy were excluded.

Ethical consideration

The ethical approval for this research was obtained from the Faculty of Health Sciences, College of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi Campus Ethics Committee. Informed consent was also sought and obtained from the subjects.

Collection of samples

About 5 ml of venous blood was collected after overnight fasting of 12 hrs in all the subjects for estimation of serum levels of total cholesterol, HDL, LDL, VLDL and triglycerides. Samples were allowed to clot and separation performed by centrifugation at 5000rpm for 5minutes.

Questionnaires were used to obtain data such as age and menstruation status of subjects.

Estimation of total cholesterol (TC)

Total cholesterol was determined using the method as described by Roeschlau *et al.*, (1974). This is essentially an enzymatic end point method.

Procedure: About 10µl of distilled water, cholesterol standard and serum were pipetted into the tubes labelled reagent blank, standard and sample respectively and 1000µl of reagent pipetted into each of the tubes. The tubes were mixed and incubated for 5minutes at 37°C. The absorbance of the sample (A_{sample}) and standard (A_{standard}) were measured against the reagent blank within 60minutes at 500nm wavelength.

Calculation

$$\text{TC concentration (mmol/l)} = \frac{A_{\text{sample}} \times \text{concentration of standard (5.09mmol/l)}}{A_{\text{standard}}}$$

Estimation of triglycerides

The enzymatic method as described by Tietz (1990) was adopted in the estimation of triglycerides.

Procedure for triglycerides estimation

About 10µl of distilled water, triglyceride standard and serum were pipetted into the tubes labelled blank, standard and sample respectively. And 1000µl of reagent pipetted into each of the tubes which were mixed and incubated at 37°C for 5minutes. The absorbance of sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 60minutes at 500nm wavelength.

Calculation

$$\text{Triglyceride concentration (mmol/l)} = \frac{A_{\text{sample}} \times C_{\text{Standard}} (2.19\text{mmol/l})}{A_{\text{standard}}}$$

Estimation of high density lipoprotein cholesterol (HDL- C)

The estimation of HDL was performed using the method as described by Burstein *et al.*, (1980). This is principally a combination of phosphotungstate precipitation and enzymatic method.

Procedure for HDL estimation

Stage 1: precipitation

About 0.2ml of the subject's sample was pipetted into the respective tubes and 0.5ml of reagent (A) pipetted into each of the tubes. The tubes were thoroughly mixed and allowed to stand for 10minutes. Centrifugation was done at 4000rpm for 10minutes and the supernatant was then carefully collected.

Stage 2: colorimetry

About 50µl of HDL-cholesterol standard, sample supernatant and distilled water was pipetted into the tubes labelled standard, sample and reagent blank respectively and 1.0ml of reagent (B) pipetted into each of the tubes. The tubes were thoroughly mixed and incubated for 10minutes at 37°C. The absorbance of the standard and sample was measured at 500nm against the blank.

Calculations

Concentration of HDL cholesterol (mmol/l): $A_{\text{sample}} \times C_{\text{Standard}} (1.36 \text{ mmol/l})$

A_{standard}

Estimation of low density lipoprotein cholesterol (LDL-C)

The method of Assman *et al.*, (1984) was adopted. This is a combination of polyvinyl sulphate precipitation and enzymatic method.

Procedure of LDL estimation

Stage 1: precipitation

About 0.2ml of subjects' sample was pipetted into the respective tubes and 0.2ml of reagent (A) pipetted into each of the tubes. The tubes were thoroughly mixed and allowed to stand for 15minutes at room temperature. Centrifugation was done at 4000rpm for 15minutes and the supernatant was then carefully collected.

Stage 2: colorimetry

About 20µl of distilled water, cholesterol standard and sample supernatant was pipetted into the tubes labelled reagent blank, standard and sample respectively and

1.0ml of reagent (A) pipetted into each of the tubes. The tubes were thoroughly mixed and incubated for 10minutes at 37°C. The absorbance of the standard and sample was measured at 500nm against the blank.

Calculations

The cholesterol concentration in the supernatant ($C_{\text{Supernatant}}$) in mmol/l was calculated using the following general formula:

$$\frac{A_{\text{Sample}} \times C_{\text{standard}} (5.18\text{mmol/l})}{A_{\text{Standard}}} = C_{\text{Supernatant}}$$

The LDL cholesterol (mmol/l) in the sample was calculated as follows:

LDL cholesterol concentration = Total cholesterol – cholesterol in supernatant

Estimation of very low density lipoprotein cholesterol (VLDL-C)

VLDL-C was estimated using the method as described by Friedwald *et al.*, (1972):

VLDL-C in sample (mmol/l) = Total cholesterol – (HDL-C + LDL-C)

Statistical analysis

Data generated from the study was subjected to statistical analysis using student t-test and ANOVA and presented in table and bar charts. Values were deemed significant at $p < 0.05$.

Results

In Table 4.1, the mean levels of serum triglycerides and LDL were significantly higher in menopausal women compared with the premenopausal group ($p < 0.05$) while there was no significant difference in the mean values of total cholesterol, HDL and VLDL cholesterol between premenopausal and menopausal women ($p > 0.05$).

Fig 4.1 shows relationship of the levels of the various lipid parameters with the duration of menopause. There was a progressive increase in the mean levels of total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol with duration of menopause while the levels of HDL-cholesterol decreased with duration of menopause.

Fig 4.2 shows relationship of the levels of the various lipid parameters with the various age categories of the menopausal subjects. There were no significant variations among the various age categories of the menopausal subjects and the levels of the lipid parameters studied ($p > 0.05$).

Table 4.1 Comparison of lipid profile in premenopausal and menopausal women

Parameters	pre-menopausal Mean ± SD	menopausal Mean ± SD	t-value	p-value
Total cholesterol	3.73 ± 0.96	3.96 ± 1.85	0.767	0.445
Triglycerides	1.27 ± 0.54	1.54 ± 0.67	2.115	0.037*
HDL-C	0.37 ± 0.66	0.25 ± 0.40	1.082	0.282
LDL-C	2.37 ± 1.06	3.14 ± 1.76	2.632	0.010*
VLDL-C	0.30 ± 0.23	0.32 ± 0.18	0.620	0.537

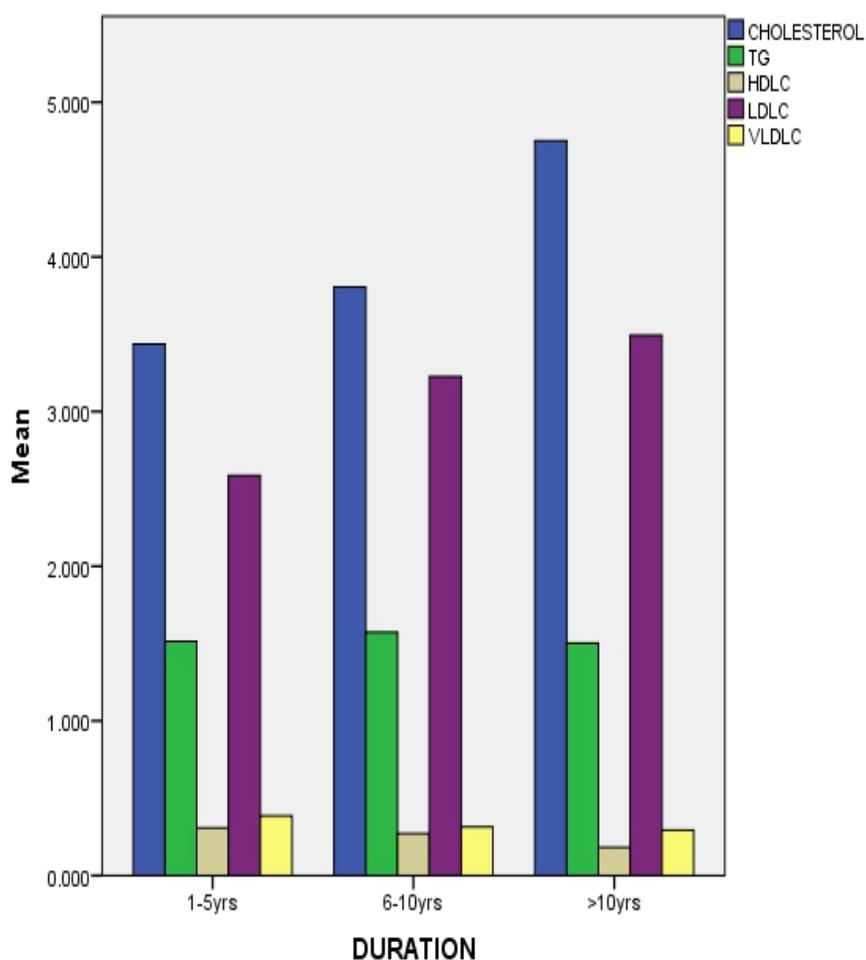


Fig. 4.1 Relationship of the levels of the various lipid parameters with the duration of menopause

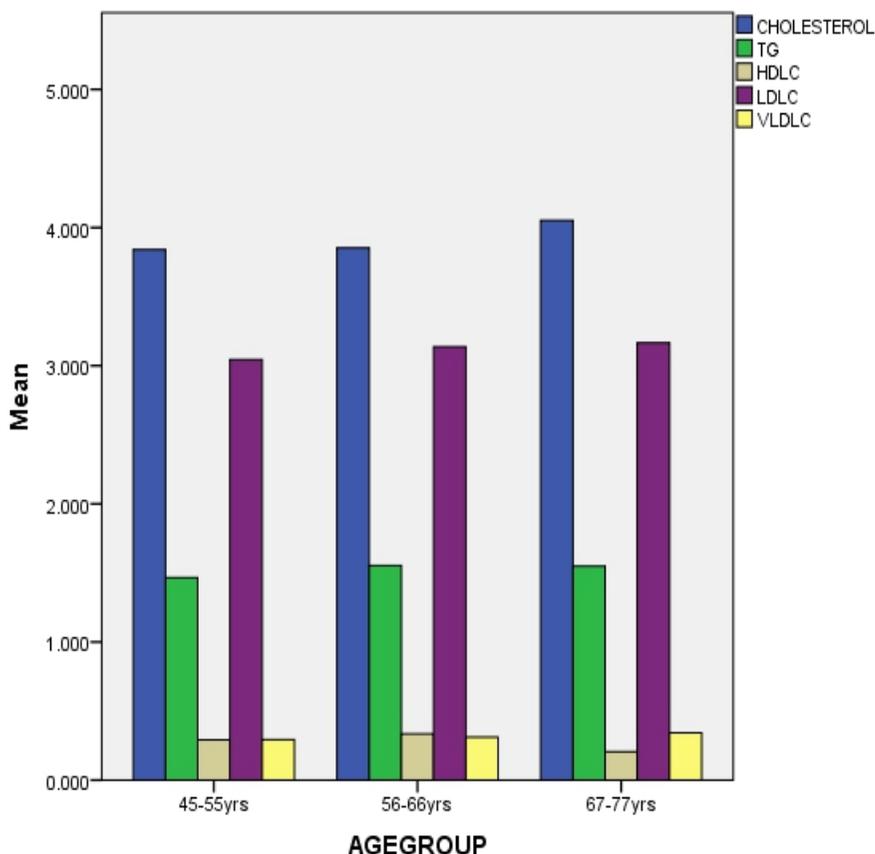


Fig. 4.2 Relationship of the levels of the various lipid parameters with the various age categories of the menopausal subjects

Discussion

One of the major causes of death among menopausal women is cardiovascular disease which accounts for nearly 53% of all deaths in women over 50 years of age (Alfonso *et al.*, 2003). Postmenopausal estrogen deficiency, hypertriglyceridemia, dyslipoproteinemia and advanced age have been described as risk factors for atherosclerosis (Narain *et al.*, 2008) and have been associated with increased coronary heart disease (CHD) risk in women (Stevenson *et al.*, 1993).

In the present study, mean levels of serum triglycerides (TG) and low density lipoprotein cholesterol (LDL cholesterol) showed a statistically significant increase in menopausal women compared with the pre-menopausal subjects ($P < 0.05$) while there was no significant difference in the mean values of serum high density lipoprotein cholesterol (HDL cholesterol), very low density lipoprotein cholesterol (VLDL cholesterol) and total cholesterol ($P > 0.05$). This correlates with previous studies by

Usoro *et al* (2006) who found statistically significant increase in levels of serum LDL cholesterol and triglycerides after menopause and that of Igweh *et al* (2005) that observed statistically significant increase in levels of serum LDL cholesterol after menopause. However, in contrast to this present study, they also found statistically significant decrease in the mean value of HDL cholesterol after menopause. This may be related to differences in sample sizes.

Increased serum triglycerides and LDL-cholesterol levels indicated in the results may be due to estrogen related decrease in activity of lipoprotein lipase (LPL) after the loss of ovarian function as observed by Stevenson *et al* (1993), Wild *et al* (1995) and Thomas *et al* (1998).

The concomitant increase in the levels of serum triglycerides and LDL-cholesterol in the menopausal group as shown in this present study could be attributed to the fact that LDL particles are formed as VLDL particles lose triglycerides through the action of lipoprotein lipase (LPL) as proposed by Warnick *et al* (1990) who had earlier reported a correspondence between higher triglyceride levels and higher levels of LDL particles. Arca *et al* (1994) had also reported that decrease in estrogen secretion with the cessation of ovarian function probably contribute to higher LDL-cholesterol level in menopausal women as estrogen increases hepatic synthesis of LDL-cholesterol receptor for Apo- β 100 resulting in increased LDL cholesterol uptake and therefore decreases circulating LDL levels. Thus, its deficiency results in rise in LDL cholesterol in menopausal women (Wild *et al.*, 1995). LDL particles and high levels of triglycerides in the bloodstream have been linked to atherosclerosis and, by extension, the risk of heart disease and stroke (Nelson and Cox, 2002). Hence, our findings suggest that premenopausal women have a less risk of atheroma-formation than their menopausal counterparts as proposed by Alfonso *et al* (2003).

Furthermore, there was a significant variation between duration of menopause and the levels of the lipid parameters studied ($p < 0.05$). This agrees with the findings of Simpson and Davis (2001) and Maulik *et al* (2012). Since many of the factors affecting serum lipid profile were excluded in our work, the relation between duration of menopause and the lipid profile status could be attributed to progressive depreciation in estrogen levels occurring after menopause as observed by Mumford *et al.* (2010). Reduced dietary cholesterol absorption may contribute to the hypocholesterolemic effect of estrogen (Karjalainen *et al.*, 2000) and may therefore account for the modest increases in the levels of serum total cholesterol after menopause.

Estrogen related decrease in the HDL cholesterol concentration after menopause may be due to a decrease in the cholesterol content in the large, cholesterol-rich HDL₂ sub-fraction, likely attributed to augmented production rates of HDL apoA-1 with no changes or mild increases in HDL

apoA-1 removal rates (Lamon-Fava *et al.*, 2006). High concentrations of HDL have cardio-protective value (Toth and Peter, 2005) while in contrast, low level of HDL particles is independently associated with atheromatous disease progression in the arteries. Hence, our findings suggest that the risk of cardiovascular diseases in menopausal women could be progressive with the duration of menopause.

There were no significant variations among the various age categories of the menopausal subjects and the levels of the lipid parameters studied ($p>0.05$), this suggests that age may not be directly related to the severity of lipid disorder after menopause as reported by Hardi and Irfanuddin (2007). However, Talat *et al.*, (2003) and Ochei and Kolhatkar (2008) provided empirical evidence indicating that serum cholesterol levels increase with age. Age has been said to contribute to elevated lipid parameters (Estari *et al.*, 2000). This may be associated to the progressive decrease in growth hormone (GH) secretion, which occurs with normal aging as observed by Corpas *et al.*, (1993). Growth hormone has an important role in cholesterol homeostasis (Angelin and Rudling, 1994) both by modulating the expression of- hepatic LDL receptors (Parini *et al.*, 1995) and by controlling the activity of cholesterol 7 α -hydroxylase (C7 α OH) (Rudling *et al.*, 1997). Joanna *et al.* (2011) had also reported age-mediated reduction in 5' adenosine monophosphate-activated protein kinase (AMPK) signaling which results in increased intramyocellular lipids. But Hardi and Irfanuddin (2007) reported that correlation between age and the alteration in the pattern of lipid profile is dependent on obesity and gender. Hence, the contrast in this present study could be attributed to the fact that the effect of age on the lipid profile status in the menopausal subjects was not investigated in relation to their body mass index or other relevant anthropometric measures.

Conclusion

Menopause is associated with altered serum lipid profile and thus an independent risk factor for developing cardiovascular diseases. The findings from this present study show that cardiovascular risk factors like LDL-cholesterol and triglycerides were significantly increased after menopause and progressively increase with the duration of menopause. The data further suggest that age may not be directly related to the severity of lipid disorders in menopausal women. Hence, menopause is associated with potential adverse changes in lipid profile status, independent of any direct effects of aging. These changes may in part explain the increased incidence of coronary heart disease seen in menopausal women.

Recommendations

In menopausal women, specific health education strategies are needed in order to prevent the incidence of cardiovascular diseases. The present study does not show the exact cause of altered serum lipid profile after menopause hence, inclusion of estrogen evaluation may help in further research. We recommend base-line and frequent determinations of lipid profile in menopausal women. This may help in the management and control of cardiovascular disorders and other pathological conditions associated with alterations in lipid profile in menopausal women.

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