Magna Scientia Advanced Research and Reviews

eISSN: 2582-9394

Cross Ref DOI: 10.30574/msarr

Journal homepage: https://magnascientiapub.com/journals/msarr/

(RESEARCH ARTICLE)

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Evaluation of serum apolipoprotein concentrations and atherogenic indices in menopausal and premenopausal women in Nkwelle-Ezunaka

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Magna Scientia Advanced Research and Reviews, 2024, 12(01), 159–165

Publication history: Received on 13 August 2024; revised on 08 October 2024; accepted on 11 October 2024

Article DOI: https://doi.org/10.30574/msarr.2024.12.1.0150

Abstract

Background of study: The hormonal change in menopause affects lipid metabolism which could lead to dyslipidaemia, a risk factor for cardiovascular disease. Aim of study: To evaluate the levels of apolipoprotein A1, apolipoprotein B, Castelli risk index-1, Castelli risk index-11, atherogenic index of plasma and atherogenic coefficient in menopausal and premenopausal women.

Materials and Methods: In this cross-sectional study ninety females were selected which consisted of 45 premenopausal, and 45 menopausal women using simple random sampling technique. Levels of apolipoprotein A1 and B were determined spectrophotometrically using enzyme linked immunosorbent assay (ELISA). Atherogenic indices were calculated with their respective formulas.

Results: There was no significant difference in the mean levels of apo B (134.83±28.90 vs 130.73±30.55; p>0.05), apo A1, atherogenic index of plasma, Castelli risk index-1, Castelli risk index-11 and atherogenic coefficient in the menopausal women compared with the premenopausal women but the mean values of both groups were outside the normal range of apo B, Castelli risk index-1, atherogenic index of plasma and atherogenic coefficient.

Conclusion: Apo B, atherogenic index of plasma, Castelli risk index-1 and atherogenic coefficient are better indicators of cardiovascular risk.

Keywords: Apolipoprotein B; Atherogenic indices; Cardiovascular disease; Metabolic syndrome; Dyslipidaemia; Menopause; Premenopause

1 Introduction

Cardiovascular disease is a leading cause of death globally [1] responsible for 20.5 million deaths in 2021 alone which is roughly one third of all global deaths[2]. Prior to menopause men are more at risk of CVD than women[3] due to the cardioprotective roles of estrogen[4]. Loss of ovarian function during menopause and subsequent estrogen deficiency is believed to promote CVD in women[5]. This could be due to reduction in the use of LDL-C to produce endogenous estrogen resulting to dysregulated lipid metabolism (dyslipidaemia)[6] a major risk factor for the development of cardiovascular diseases[7] .

Dyslipidaemia is conventionally evaluated with total cholesterol, high density lipoprotein, low density lipoprotein, triglyceride and very low-density lipoprotein levels[8] though lipid ratios such as atherogenic index of plasma, atherogenic coefficient and coronary risk index are said to perform better than individual lipids in predicting

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cardiovascular risk[9].These lipid ratios measure the relationship between proatherogenic and anti-atherogenic lipid factors. Atherogenic index of plasma, atherogenic coefficient, Castelli risk index-1 and Castelli risk index 11 are calculated with lipid parameters.

On the other hand apo B and apo-A1 are major apolipoproteins involved in lipid metabolism[10]. Apo B is an essential component of all atherogenic lipids[11] while apolipoprotein-A1 is a major component of antiatherogenic lipids[12]. They are considered useful and superior to total cholesterol and triglyceride for predicting cardiovascular risk[13].This study analysed the cardiometabolic status of menopausal and premenopausal women using atherogenic indices, apo A1 and apo-B.

2 Materials and Methods

2.1 Study area

This research was conducted in Nkwelle-Ezunaka, Anambra state. Nkwelle-Ezunaka is one of the five towns in Oyi local government area of Anambra state[14], located about 8.5 kilometers northeast of Onitsha, Anambra state. It is bordered by nine neighbouring towns: Nteje and Umunya by the east, Nsugbe and Umueri by the north, Onitsha and Obosi by the west and Nkpor, Ogidi and Ogbunike by the south. Nkwelle-Ezunaka has a vast land rich in farming and is a fast-developing suburban area in Nigeria.

2.2 Study design

A cross-sectional study designed to assess cardio metabolic disorders in menopausal women. A total of 90 female subjects within the age range of 19 to 65 years were recruited for the study using random sampling techniques. This included 45 menopausal women and 45 premenopausal women. A random pick of 2 areas was made with an average of 45 individuals mobilized for the study from each selected area. Participants were interviewed via structured questionnaires and physical assessment.

2.3 Sample size

Sample size was determined using Daniel[15] sample size formula as described by[16].

$$N = \frac{z^2 p(1-p)}{d^2}$$

N= sample size, z = confidence interval, p= expected prevalence or proportion and d = precision. Here confidence interval of 1.96 and precision of 0.05 was used and menopausal prevalence of 3.96%.

N = (1.96)^2 X 3.96% (1-3.96%)/0.05^2

N = 3.8416 X 3.96/100 (1-3.96/100)/0.05^2

N=3.8416 X 0.0396(1-0.0396)/0.0025

N = 3.8416 X 0.0396(0.604)/0.0025

N= 3.8416 X 0.0396 (241.6)

N= 3.8416 X 9.567

N = 36.7

N= 36.7

N = 37

A minimum sample size of 37 was arrived at using menopausal prevalence rate of 3.96% as cited by Ezeugwunne[17] but a total of 90 subjects were recruited for the study.

2.4 Ethical consideration

The ethical approval was obtained from Ethics Committee, Nnamdi Azikiwe Teaching Hospital, Nnewi (NAUTH/CS/66/VOL.16/VER.3/306/2021/080). The study participants were enlightened on the purpose of the study and allowed to choose to volunteer verbally.

2.5 Inclusion criteria

Apparently healthy premenopausal and menopausal females within the age range of 19 to 65 years.

2.6 Exclusion criteria

Individuals on hormonal treatments and those outside the age range of 19 to 65 years. The exclusion was done after reviewing filled questionnaire.

2.7 Sample collection and storage

Five milimeters of fasting blood sample was collected from participants via venipuncture. Two (2) mls was dispensed into fluoride oxalate tube and centrifuged at 2500 revolution per minute to obtain plasma for glucose estimation while three (3) mls was dispensed into plain tube, allowed to clot for 2 hours at room temperature. This was centrifuged at 3000rpm to obtain serum for apolipoproteins, lipid profile, fasting plasma glucose and insulin assays. Samples were frozen at 4℃ prior to analysis.

2.7.1 Determination of apolipoprotein A1 levels

Apo A1 was assayed using the sandwish ELISA method as described by Zhong et al[18].

2.7.2 Principle for the determination of apolipoprotein A1

Apolipoprotein A1 reacts with supersensitized apolipoprotein A1 antibody to generate immune complex (turbid) measured at 340nm. The degree of turbid change is proportional to the apo A1 levels in the samples.

2.7.3 Procedure for apolipoprotein A1 determination

The enzyme-linked immunosorbent assay (ELISA) assay is a time and temperature sensitive method. All reagents and samples were brought to room temperature before use. Two hundred and twenty-five (225) µl of Reagent 1 (R1) was added to the anti-apoAI coated micro well followed by 2 µl of the sample. The mixture was mixed thoroughly but gently and incubated for 5mins at 37ºC. The absorbance was read at 340nm. Seventy-five (75) µl of Reagent 2 (R2) was added to each well and incubated for 5 minutes. The absorbance was read.

2.7.4 Determination of apolipoprotein B levels

Apo B was assayed using the sandwish ELISA method as described by Fonseca et al[19].

2.7.5 Principle

Apolipoprotein B reacts with supersensitized apolipoprotein B antibody to generate immune complex (turbid) measured at 340nm. The degree of turbid change is proportional to the apo B levels in the samples.

2.7.6 Procedure

The ELISA assay is a time and temperature sensitive method. All reagents and samples were brought to room temperature before use. Two hundred and twenty five (225) µl of R1 was added to the anti-apoB coated micro well followed by 2 µl of the sample. The mixture was mixed thoroughly but gently and incubated for 5mins at 37ºC. The absorbance was read at 340nm. Seventy five (75) µl of R2 was be added to each well and incubated for 5 minutes. The absorbance was read.

2.8 Assessment of atherogenic indices

The different athrogenic indices of each participant was calculated according to the method described by Igharo[20].

- Atherogenic index of plasma (AIP) equals to logarithm of the ratio of TG and HDL (Log (TG/HDL).
- Castelli risk index I (CRI-II) equals to the ratio of total cholesterol and HDL (TC/HDL).
- Castelli's risk index II (CRI-II) equals to the ratio of LDL and HDL (LDL/HDL).

- Atherogenic coefficient (AC) equals to the ratio of non HDL cholesterol to HDL cholesterol

2.9 Statistical Analysis

Statistical package for social sciences version 26.0 was used for data analysis. Data were presented as mean±SD and analysed using the student's t-test. Results at $p<0.05$ was deemed significant. Correlation studies was performed using the Pearson's correlation coefficient and Levene test of variance.

3 Results

There was no significant difference in the means levels of apolipoprotein B, apolipoprotein A1, atherogenic index of plasma, Castelli index I , Castelli risk index-II and atherogenic coefficient in the test subjects compared with the control subjects (134.83 ± 28.90 vs 130.73 ± 30.55; $p>0.05$), (183.84 ± 15.07 vs 181.16 ± 15.48; $p> 0.05$), (0.37 ± 0.12 vs 2.98 ± 0.75; $p<0.05$) (4.74 ± 0.80 vs 4.39 ± 0.89 ; $p<0.05$) (3.24 ± 0.77 2.98 ± 0.75 $p<0.05$) and (3.71 ± 0.80 vs 3.71 ± 0.80; $p<0.05$) in the test subjects compared with the control respectively.

Levels of Apo B, Apo A1, AIP, CRI-I, CRI-II and AC in control and test subjects (mean ± SD)

Table 1 Comparison of the mean levels of apolipoprotein A1, apolipoprotein B, atherogenic index of plasma, Castelli risk index I , Castelli's index ii and atherogenic coefficient in premenopausal (control) and menopausal women (test) (mean ± SD).

Parameters	Test	Control	t-test	p-value
APO B	134.83 ± 28.90	130.73 ± 30.55	-0.654	0.515
APO A1	183.84 ± 15.07	181.16 ± 15.48	-0.831	0.408
AIP	0.37 ± 0.12	0.38 ± 0.12	0.064	0.949
CRI-I	4.74 ± 0.80	4.39 ± 0.89	-1.970	0.052
CRI-II	3.24 ± 0.77	2.98 ± 0.75	-1.403	0.164
AC	3.71 ± 0.80	3.71 ± 0.80	-1.860	0.066

Statistically significant at $p<0.05$; APO = apolipoprotein, AIP = atherogenic index of plasma, CRI = castelli risk index, AC = atherogenic coefficient.

4 Discussion

The increasing prevalence of cardiovascular events such as coronary heart disease, myocardial infarction, stroke and peripheral vascular disease is majorly due to dyslipidaemia [21] . However, dyslipidaemia, the most crucial modifiable risk factor[22] can be assessed using lipid ratios in line with lipid parameters even when lipid profiles are apparently normal (21) and apo B which is also an early indicator and a more accurate predictor of cardiovascular risk than LDL-cholesterol and non-high density lipoprotein cholesterol[23,24]. Our study shows no significant difference in atherogenic index of plasma, Castelli risk index-1, Castell risk index-11 and atherogenic coefficient when the data of both groups were compared. Moreover using the international standard reference range of atherogenic indices to compare the mean variables of both groups, atherogenic index of plasma, Castelli risk index-1 and atherogenic coefficient were off the normal limits. This suggests a high prevalence of dyslipidaemia in both premenopausal and menopausal women.

Our findings contradict those of Reddy and Chandala [25] and Ranjit, Guntuku and Pothineni [26] who reported a significant increase in the atherogenic indices of the menopausal women compared with the premenopausal women.

There was also no significant difference in apo-B and apo-AI levels in both groups which did not tally with the findings of Ogbodo et al [27] and Swapnali, Kisan and Muritthy [28] who reported higher levels of apo-B and lower levels of apo-A1 in the menopausal women compared with the premenopausal women. Though the levels of apo-B in both menopausal and premenopausal women didn't fall within the normal range.

This disparity could be due to smaller sample size but comparing the values of atherogenic indices, apolipoprotein B and apolipoprotein A1 levels with the normal ranges it could also be due to prevailing metabolic syndrome in both

groups as suggested by the findings of Grishma[29] after analysing WHO data from NHANES from 2011-2016 for the prevalence of metabolic syndrome and he found that the prevalence of metabolic syndrome in women increased from 31.7 to 36.6% and from 16.2 to 21.3 in those aged 20 -39years. This proposition aligns with the discoveries of Sharma[30] who found 59.4% prevalence of MS in premenopausal and 65.7% in menopausal women and that of Pandey et al, with premenopausal and menopausal women having 45% and 55% prevalence of metabolic syndrome respectively[31].

5 Conclusion

This study highlights the important role, atherogenic index of plasma, Castelli risk index-1, atherogenic coefficient and apo B play in the detection of individuals at high risk of cardiovascular disease irrespective of their menopausal status. It suggests a high prevalence of dyslipidaemia in premenopausal and menopausal women. Therefore, measures need to be reinstated to improve the metabolic health of women through frequent monitoring of lipid profile, lipid ratios and apo B. There is also need for public health education targeted at empowering women with metabolic health information and practical strategies for improved metabolic health outcome.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

If studies involve use of animal/human subject, authors must give appropriate statement of ethical approval. If not applicable then mention 'The present research work does not contain any studies performed on animals/humans subjects by any of the authors'.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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