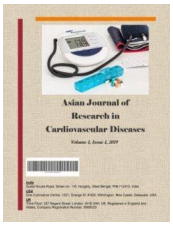

Asian Journal of Research in Cardiovascular Diseases

2(2): 29-41, 2020; Article no.AJRCD.58744

Comparative Study of Hormonal Contraceptives on Biochemical and Atherogenic Parameters of Women in Rivers State University Community, Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author HB designed the study and wrote the protocol. Author IE performed the statistical analysis wrote the first draft of the manuscript. Author AMA managed the analyses of the study. All authors read and approved the final manuscript.

Article Information

Editor(s):
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(3) Titik Kurniawati, Akademi Kebidanan Abdi Husada Semarang, Indonesia.
Complete Peer review History: http://www.sdiarticle4.com/review-history/58744

Original Research Article

Received 19 May 2020
Accepted 24 July 2020
Published 05 August 2020

ABSTRACT

Aim: To compare the effect of oral and injectable hormonal contraceptives on lipid parameters, calcium, phosphate and some atherogenic indices among women in Rivers Sate University community, Port Harcourt, Nigeria.
Study Design: One hundred and fifty (150) women between the ages of 18 - 44 years in Rivers State University attending family planning clinics within Port Harcourt, Rivers State that are not pregnant and non-lactating were recruited. Of the 150 women recruited, 50 women were on injectable depo provera contraceptives, 20 of the women were on injectable noristerat contraceptives while 30 of them were on combined oral combined contraceptives. Fifty (50) women not on contraceptives were used as control. These control women did not use oral or injectable

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contraceptives whether implants, or any other contraceptive method excluding barrier methods like condoms.

Methodology: A randomized controlled design with samples collected randomly within the University community was used in this study. A well-structured questionnaire was given to all the intending participants to obtain demographic information, medical history and pattern of lifestyle after getting consent. Women included were those attending family planning clinics on contraceptives for at least 3 months, not lactating, not pregnant, without history of hypertension, cardiovascular disorders, osteoporosis, diabetes mellitus or smoking. However, women that were lactating, pregnant, not attending family planning clinic or with history of hypertension, cardiovascular disorders, osteoporosis, diabetes mellitus or that are smoking were excluded. Five millilitres **(**5mls) of fasting whole blood samples was collected into plain bottles. The collected whole blood samples were allowed to clot and later retracted and spun at 400rpm for 10 minutes to obtain serum. The laboratory analysis of calcium, phosphate, and lipid parameters were performed using Standard procedure BS-800 Mindray chemistry analyzer. LDL-C was calculated using Frieldewald equation. Atherogenic Index of Plasma (AIP) was estimated using the equation: AIP=[Log(TG/HDL-C)] while Non-High Density Lipoprotein-C (NHDL) was calculated using the equation NHDL-C=TC–HDL-C.

Results: The results obtained showed higher and lower values levels of LDL-C and HDL-C respectively in women using oral contraceptives. Lower values of calcium and phosphate were also seen in women using oral contraceptives. In addition, AIP and NHDL-C values were higher in women using oral contraceptives compared to control women and women on injectables contraceptives especially Depo provera. On the other hand, women on injectables contraceptives especially depo provera showed lower and higher values levels of LDL-C and HDL-C respectively. Higher values of calcium and phosphate were also seen. In addition, AIP and NHDL-C values were lower in women using injectables contraceptives compared to control women and women on oral contraceptives. More so, results seen at the different age interval showed that women using contraceptives at age 18-26 years had lesser risk for cardiovascular disease compared to age 27 - 35 years. However, age 36 -44 years indicated significantly higher values of atherogenic lipids like LDL-C and atherogenic indices like NHDL-C. Significantly lower values of calcium and phosphate were also seen within this age bracket.

Conclusion: Results obtained demonstrated that injectable contraceptives exerted more beneficial effect on lipid metabolism and cardiovascular disease while oral contraceptives had more bone mineralization effect. Again, when age interval were considered, women at age 18-26 years had low risk of dyslipidaemia and mineralization of bones were not affected compared to age 27-35 years while age 36-44 years were at risk of cardiovascular diseases and enhanced bone mineralization tendencies.

Keywords: Oral contraceptives; injectables contraceptives; biochemical; atherogenic indices; lipid parameters; calcium; phosphate.

ABBREVIATIONS

CW = Control Women
HCs = Hormonal Contraceptives
ICs = Injectibles Contraceptives
OCs = Oral Contraceptives
WOC = All Women on Contraceptives (WOOR + WON + WODP)
WODP = Women on Depo provera
WOI = Women of injectibles (WON + WODP)
WON = Women on Noristerat
WOOR = Women on Oral Contraceptives

1. INTRODUCTION

Contraception is being defined as any technique employed in preventing pregnancy and the technique or substance used in achieving contraception is called a contraceptive [1]. There are different forms or methods in which contraceptives are applied [1]. However, in this study the use of hormonal contraceptives (HCs) will be our focus. Usually HCs are administered in form of implants, injections or orally. The efficacy of injectables contraceptives (ICs) and oral contraceptives (OCs) have never been in doubt but side effects of both ICs and OCs have been reported in women taking such pills or medications [1,2]. Biochemical parameters of interest in this study include: cholesterol, triglyceride, high density lipoprotein and low density lipoprotein, some atherogenic indices and electrolytes such as calcium and phosphate.

Cholesterol is an unsaturated steroid and functions as precursor for the biosynthesis of bile acids, steroid hormones, and vitamin D. It is also an important structural component of cell membranes [2,3]. Generally, lipids are transported in lipoproteins such as chylomicrons, very low density lipoprotein (VLDL), Low Density Lipoprotein (LDL) Intermediate-Density Lipoprotein (IDL), and High Density Lipoprotein (HDL) [3,4]. An increased and decreased level of cholesterol is termed hypercholesterolaemia and hypocholesterolaemia respectively. Several factors have been reported to induce derangements in cholesterol ranging from lifestyle, genetic disposition, medications or drugs, underlying diseases and so on [5,6]. It has been reported that the use of HCs especially OCs increases the level of atherogenic lipids which are strongly associated with cardiovascular disorders [6,7].

Triglycerides are high-energy lipids containing both saturated and unsaturated form of fatty acids [4,6,8]. Abnormal metabolism of triglyceride is associated with coronary heart disease and other forms of cardiovascular disorders. Okere et al. [2]; Naz et al. [6], documented that the use of OCs significantly increased the level of triglycerides but such increases were not observed when ICs were investigated. However, Gali et al. [3]; Asare et al. [4], stated that the use of HCs whether oral or injectables did not induce significant difference in triglycerides level among women using HCs. It has been documented severally that abnormalities in LDL metabolism usually lead to atherosclerosis [4,6]. In a study carried out by [Gali et al. [3]; Asare et al. [4], it was reported that the use of OCs caused significant increase in LDL-cholesterol among women and that ICs did not cause any significant difference. However, Adaja et al. [9], reported a significant lower values of LDL-cholesterol in women on both OCs and ICs compared against women not on contraceptives.

HDLs are sometimes referred as “scavengers” in lipid metabolism [5]. Clinically, significantly lower levels of HDL are associated with cardiovascular diseases specifically coronary heart disease as observed in tangier disease and familial hypoalphalipoproteinaemia [4,5]. In a study performed by Gali et al. [3], it was observed that the use of injectables HCs did not cause a significant difference in HDL among women but a significant increase in HDL-cholesterol was seen in women on OCs. However, Okere et al. [2], reported a significant increase in HDL-cholesterol in women on injectables. Okere et al. [2], further documented that the use of combined OCs did not cause any significant difference in plasma HDL-C levels. More so, Asare et al. [4], demonstrated that HCs whether OCs or ICs did not induce any significant difference in HDL-cholesterol level in women.

Atherogenic indices assist strongly in predicting CVD risk mostly when lipids’ absolute values are not markedly deranged [7,8]. Atherogenic indices considered in this study include Atherogenic Index of Plasma (AIP) and Non-High-density lipoprotein cholesterol (NHDL-C). AIP is a very vital index because it compares the size of HDL-C and LDL-C particles and therefore could serve as an indicator of the lipoprotein atherogenicity [8,9,10]. An increase in AIP predicts high risk of CVD. AIP values in humans of <0.11, 0.11-0.21 and >0.21 indicate low, intermediate and high risk of CVD respectively [8,10]. NHDL-C indicates the fractions of the atherogenic lipoproteins that make up cholesterol [11]. NHDL-C of 3.4 - 4.0 mmol/L indicates no CVD risks, <3.4 mmol/L and value of 4.9 - 5.6 mmol/L indicates moderate risk of CVD, <2.6 and >5.7 indicate very high risk of CVD [11]. NHDL-C is derived by the removal of the high density lipoprotein (HDL-C) fraction from total cholesterol (TC) [11]. It has been reported that in developing countries CVD is expected to rise above 18 million by 2020 with deaths affecting age bracket of 15 – 59 [7,12]. As reported by Ugwuja et al [12]; Ukpabi & Uwanurochi [13], most unexpected deaths of a medical origin in Nigeria are due to CVD and the prevalence of CVD among young people is becoming medical concern with an average of 17.6%. More so, the assessment of lipoproteins and other risk factors for CVD in younger population and women at child-bearing age has become important since metabolic changes such as atherosclerosis has been reported to begin in childhood and adolescent without sign of CVD risks [14,15,16]. Therefore, early detection of CVD risks could present a lot of time to slow down metabolic changes that results in obesity and dyslipidaemia especially in women at child-bearing age.

Calcium is one of the most abundant electrolytes in the body and functions as coenzyme for coagulation factors, excitability and release of neurotransmitters, mineralisation of bones and so on [1,17]. Plasma calcium control relies on adequate supply of calcium in diet, vitamin D, functional Intestine, thyroid and parathyroid gland and kidneys [18]. An increased plasma level of

calcium is termed hypercalcaemia while reduced level is termed hypocalcaemia. Derangements in plasma calcium could be as a result of drugs and chemicals, vitamin D deficiency or toxicity, chronic renal failure, severe hypomagnesaemia or hypermagnesaemia, acute pancreatitis and elevated or decreased calcitonin levels [18]. Studies have also reported that plasma calcium levels are affected among women using HCs. Ganero et al. [19]; Cobb et al. [20], reported in their respective work that oral HCs play vital role in protecting bone mass density by increasing calcium absorption into bones with resultant hypocalcaemia. Obisesan et al. [1], stated that OCs increases albumin concentration in the plasma. Albumin, as major transporter of calcium in the plasma, could enhance the increase turnover of calcium from plasma to bone resulting in reduced plasma calcium concentration. Dante et al. [21], reported that injectable HCs reduce plasma phosphate level thereby triggering a significant increase in plasma calcium level especially when compared to OCs. Dante et al. [21], further reported that HCs (Injectables and oral) stimulates the release of calcitriol which in turn stimulates increase intestinal absorption of calcium but calcium or phosphate plasma level were not affected. However, Hartland et al. [22], reported that the use of OCs decreases the absorption of bone minerals such as calcium and phosphate leading to osteoporosis in women in their reproductive age.

Phosphate is a major intracellular anion and has several functions such as the formation of cell membrane and generation of energy in the form of ATP [21,23]. Increased plasma level of phosphate is termed hyperphosphataemia while a significant decrease is termed hypopho-sphataemia. Disorder of phosphate could be due to vitamin D insufficiencies or toxicity, hypothyroidism or hyperthyroidism, acute or chronic renal failure, low or high phosphate intake and increase tissue breakdown [23]. Studies have reported that oral or injectable HCs also induce derangements in plasma phosphate levels. Hartland et al. [22], reported that the use of OCs decreases the absorption of bone minerals such as calcium and phosphate leading to osteoporosis in young women. Moller et al. [24], reported that HCs stimulates the release of calcitriol which in turn stimulates increase intestinal absorption of calcium and phosphate but their plasma levels were not affected. Akinloye et al. [25], reported that oral HCs reduce plasma phosphate among women in reproductive age. Adesiyan et al. [26]; Hammed et al. [27], also reported in their respective studies that oral as well as injectable HCs induce hypophosphataemia among women in their reproductive age, playing vital role in protecting bone mass density by absorbing calcium, phosphate and magnesium into bones from the plasma.

Oral hormonal contraceptives consisting mainly of estrogen and progesterone and injectable contraceptives mostly consisting of progesterone have been reported to exert some varying biochemical changes in women in their reproductive age as well as undesirable side effects [1,2,4]. Therefore, in this work, the comparative effect of oral contraceptives and injectable contraceptives on biochemical parameters such as calcium, phosphate, lipid parameters as well as some atherogenic indices will be considered. This study will also consider the influence HCs on different age intervals among women in their reproductive years.

2. MATERIALS AND METHODS

2.1 Subjects Characteristics

One hundred and fifty (150) women between the ages of 18-44 years in Rivers State University attending family planning clinics within Port Harcourt, Rivers State were recruited. The study design was randomized controlled study with samples collected randomly within the University community. Of the 150 women, 50 were not on HCs and were used as control. These control women did not use oral or injectable contraceptives whether implants, or any other contraceptive method excluding barrier methods like the use of condoms while 100 women on HCs (oral or injectables) for at least a minimum of 3 months were used as test subjects. Of the 100 women on contraceptives, 30 were on OCs while 70 were on ICs. Of those on ICs, 50 were on Depo-provera while 20 were on Noristerat.

2.1.1 Inclusion and exclusion criteria

A well-structured questionnaire was given to all the intending participants to obtain demographic information, medical history and pattern of lifestyle after getting consent. Women included in this study were women attending family planning clinics on contraceptives for at least 3 months, not lactating, not pregnant, without history of hypertension, cardiovascular disorders, osteoporosis, diabetes mellitus or smoking. Omron digital blood pressure kit (Omron

healthcare co., Ltd, Japan) was used to check the blood pressures while fasting glucose of these women was determined using Accu-Chek glucometer (Roche, Germany). However, women that were lactating, obese, pregnant, not attending family planning clinic or with history of hypertension, cardiovascular disorders, osteoporosis, diabetes mellitus or that are smoking were excluded.

2.2 Study Area

The study was carried out in Rivers State University, Port Harcourt, South-South Nigeria. Rivers State University is located in Port Harcourt metropolis hosting over 4000 students yearly as well as over 300 family units within the main campus alone. A well functional hospital is also within the University and its (university) location permits quick access to other tertiary health-care centres. Port Harcourt is one of the largest and populous cities in Nigeria. It is also the commercial hub in southern Nigeria with several multi-national and local oil and gas companies.

2.3 Sample Collection, Preparation and Laboratory Assay

Materials used include, BS-800 Mindray chemistry analyzer, Omron digital blood pressure kit (Omron healthcare co., Ltd, Japan), plain bottles, hypodermic syringes, and centrifuge. Fasting whole blood specimens (5ml) were collected in plain bottles and were allowed to clot. The specimens were then retracted with an applicator stick and spun at 4000 rpm for 10 minutes to obtain sera which were aliquot into another set of well-labelled plain bottles. Calcium, phosphate, triglycerides, total cholesterol and High Density Lipoprotein-C were analysed using standard procedures designed by BS-800 Mindray chemistry analyzer except for LDL-C that was calculated using Friedewald equation as described by [28]. AIP was estimated using the equation: AIP=[Log(TG/HDL-C)] as described by as described by [8] while non- High Density Lipoprotein-C (NHDL) was calculated usinig the equation NHDL-C=TC–HDL-C as described by [16]. The analyses of the samples were done at the University of Port Harcourt Teaching Hospital, Port Harcourt.

2.4 Statistical Analysis

Data obtained were statistically analyzed using GraphPad Prism version 8.02 (California, USA). Descriptive statistics in form of mean and standard deviation were used while inferential statistics using one way ANOVA was employed. Multiple comparisons were done using turkey's multiple comparison tests. Statistical significant was set at P=.05.

3. RESULTS

3.1 Results of Biochemical and Atherogenic Parameters in Different Hormonal Contraceptives in Women

The values of biochemical and atherogeinc parameters were analysed using one way ANOVA with Post-Hoc done using tukey's Multiple Comparison Tests (Table 1). When calcium was analysed, the results showed a significant lower value in calcium level among women using oral contraceptives (WOOR) and women on noristerats (WON) when compared to control women (CW). More so, a significant lower value of calcium was also observed in all the women on contraceptives combined (WOC) when compared against women not on contraceptive. A significant lower value was also observed in WOOR when compared with women on Depo provera (WODP) and women on noristerat and depo provera combined (WOI). Again, a significant lower value was seen in Women on noristarat (WON) when compared against women on depo provera (WODP) at P=.05 (Table 1). When phosphate was considered, result showed significant higher value in WON compared to CW while a significant reduction were seen in WOOR, WODP and WOI compared WON at P=.05.

In addition, when total cholesterol and triglycerides were considered, significant differences were not seen in the various groups of women that used different hormonal contraceptives at P=.05. However, higher levels of TC and TG were seen in WOOR and WON respectively. In the case of HDL-cholesterol, the results obtained showed significant higher value in WODP and WOI compared to CW. Again, significantly higher values were seen in WODP and WOI when compared to WOOR. In other words, WOOR showed significant lower value in HDL-C concentration compared to WOI and WODP at P=.05. Again, significant higher values of HDL-C were seen in WODP when compared to WON and WOC. However, WON showed significant lower value in HDL-C level compared to WOI and WOC at P=.05. When LDL-C was analysed, the results showed a significant higher level of LDL-C level among WOOR, while a

significant lower value was seen in WODP when compared to CW. Also, significant increase was also observed LDL-C level in WOOR when compared with WODP, WOI, and WOC. More so, significant higher levels of in LDL-C were seen in WON and WOC when compared with WODP at *P*=.05 (Table 1).

In addition, when atherogenic markers were considered, AIP indicated significant lower value in WODP when compared to CW. In like manner, there was also a significant reduction in AIP in WODP compared to WOOR and WON. In other words, significant higher values of AIP were seen in WOOR and WON compared to WODP. Finally, when NHDL-C was considered, significant lower values were seen in WODP compared to CW. Again, significantly higher values of NHDL-C were seen in women on oral contraceptives (WOOR) and WON when compared to women on depo provera (WODP). However, WON indicated significant lower values in NHDL-C levels when to women on WOI (Table 1).

3.2 Results of Biochemical and Atherogenic Parameters in Different Age Interval of Women on Hormonal Contraceptives

When the different ages of the women recruited for this study were considered, between the range of 18-26 years, significant lower values were seen in LDL-C and NHDL while significant higher values were seen in HDL-C among these young reproductive women using contraceptives. Mores, when the age range of 27-35 years was considered, calcium and phosphate indicated significant lower values and significant higher values respectively in mid-reproductive women taking contraceptives and finally when age range of 36-44 years were considered, significant lower value was seen in calcium and phosphate while significant higher values were seen in LDL-C, and NHDL-C in women close to menopause taking contraceptives (Table 2).

4. DISCUSSION

In this research work, the effect of HCs on lipid parameters, atherogenic indices and electrolytes were investigated in women attending family planning clinic in Port Harcourt Metropolis.

The significant lower values seen in women on oral contraceptives (WOOR), women on noristerat (WON) and Women on contraceptives (WOC) compared to control women are in line with the report of Ganero et al [19]; Akinloye et al. [25]; Hameed et al. [27]. They all observed in their separate work a significant lower plasma calcium level in women on contraceptives against women not on contraceptives. However, our findings are contrary to the reports of Hartland et al. [22]; Moller et al. [24]. They reported significant higher calcium plasma level among women using HCs. In addition, Dante et al. [21] reported that injectable HCs reduce plasma phosphate level thereby triggering a significant increase in plasma calcium level especially when compared to women on OCs. Dante, et al. [21], further reported that HCs (Injectables and oral) stimulates the release of calcitriol which in turn stimulates increase intestinal absorption of calcium but calcium or phosphate plasma level were not affected. Our results also showed that women using OCs (WOOR) had lower levels of calcium compared to women on Injectables (WOI) such as depo provera and noristerat as well as lower levels of calcium in women on noristerat (WON) when compared against women on depo provera (WODP). These results are still in agreement with the reports of Dante et al. [21]. Dante et al. [21], reported higher levels of calcium in women using injectable HCs against those using OCs.

The hypocalcaemia observed among women using OCs against women using depo provera and noristerats as well as the hypocalcaemia seen in Noristerat compared to depo provera could be as a result of increase absorption of calcium from the plasma into bones due to the presence of estrogen derived from the combined oral contraceptives and Noristerat. Estrogen increases intestinal absorption of calcium into plasma by enhancing calcitriol which further stimulates mineralization of bones which is useful in preventing osteoporosis especially in menopausal or post-menopausal women. Though, noristerat is a progestin-only injectable contraceptive, unlike depo provera, noristerat is derived synthetically from estradiol (estradiol methyl ether) which is the active form of estrogen. Therefore, the hypocalcaemic effect could be as a result of minute estrogen fractions contained in noristerat. This finding support the notion of free hormone hypothesis which states that only minute free fraction of hormones has biological effect. The results obtained from this study indicates in terms of calcium mineralisation of the bone among women using contraceptives, the effect is in the order of oral contraceptives>noristerat>depo-provera contraceptives.

Table 1. Results of biochemical and atherogenic parameters in different hormonal contraceptives used by women analysed using one way ANOVA

Parameters	Calcium (mmol/L)	Phosphate (mmol/L)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	AIP	NHDL-C (mmol/L)
CW	2.50±2.96^{a}	1.25±0.34^{a}	4.30±1.15^{a}	0.82±0.46^{a}	1.61±0.42^{a}	2.34±1.09^{a}	-0.34±0.23^{a}	2.69±1.06^{a}
WOOR	2.28±0.21^{bc}	1.17±0.24^{ac}	4.40±1.16^{ab}	0.80±0.40^{ab}	1.47±0.53^{ac}	2.57±1.14^{bc}	-0.34±0.26^{ac}	2.92±1.15^{ac}
WODP	2.46±0.21^{ade}	1.21±0.31^{ace}	4.23±1.07^{abc}	0.74±0.40^{abc}	2.02±0.59^{bde}	1.86±0.86^{bde}	-0.45±0.26^{bde}	2.21±0.92^{bde}
WON	2.31±0.31^{bcfg}	1.49±0.70^{bdfg}	4.28±1.35^{abcd}	1.96±1.17^{abcd}	1.52±0.84^{acfg}	2.37±0.75^{acfg}	-0.34±0.18^{acfg}	2.76±0.79^{acfg}
WOI	2.42±0.25^{adegh}	1.29±0.47^{acehi}	4.24±1.15^{abcde}	1.09±0.69^{abcde}	1.88±0.70^{bdehi}	2.01±0.10^{adegh}	-0.42±0.25^{acegh}	2.37±0.91^{adehi}
WOC	2.38±0.25^{bcegh}	1.25±0.42^{acehi}	4.29±1.15^{abcde}	1.00±0.26^{abcde}	1.76±0.68^{acfhi}	2.17±0.98^{adfgh}	-0.40±0.25^{acegh}	2.54±1.02^{acegi}
P values	0.01	0.03	0.94	0.07	0.01	0.01	0.049	0.01
Remark	S	S	NS	NS	S	S	S	S

Calcium, LDL-C & AIP: Values in the same column with different superscripts (a, b) differ significantly when CW was compared with other groups at P=.05. Also, values in the same column with different superscripts (c, d) differ significantly when WOOR was compared with other groups at P=.05. More so, values in the same column with different superscripts (e, f) differ significantly when WODP was compared with other groups at P=.05 while values in the same column with same superscripts (g) do not differ significantly when WON was compared with other groups at P=.05 and finally values in the same column with same superscripts (h) do not differ significantly when WOI was compared with WOC group at P=.05. Phosphate, HDL-C & NHDL-C: Values in the same column with different superscripts (a, b) differ significantly when CW was compared with other groups at P=.05. Also, values in the same column with different superscripts (c, d) differ significantly when WOOR was compared with other groups at P=.05. In addition, values in the same column with different superscripts (e, f) differ significantly when WODP was compared with other groups at P=.05. Furthermore, values in the same column with different superscripts (g, h) differ significantly when WON was compared with other groups at P=.05. However, values in the same column with same superscripts (i) do not differ significantly when WOI was compared with WOC group at P=.05. TC & TG: Values in the same column with same superscripts (a) do not differ significantly when CW was compared with other groups as well as values in the same column with same superscripts (b) do not differ significantly when WOOR was compared with other groups at P=.05. Again, values in the same column with same superscripts (c) do not differ significantly when WODP was compared with other groups at P=.05. In addition, values in the same column with same superscripts (d) do not differ significantly when WON was compared with other groups at P=.05. Finally, values in the same column with same superscripts (e) do not differ significantly when WOI was compared with WOC group at P=.05. CW=control women, WOOR=women on oral contraceptives, WODP=women on Depo provera contraceptives, WON=Women on Noristarat contraceptives, WOI=Women on Injectible contraceptive (WODP+WON), WOC=All Women of contraceptives (WOOR+WODP+WON). TC=Total cholesterol, TG=Triglyceride, HDL-C=High density Lipoprotein, LDL-C=Low density Lipoprotein, AIP=Atherogenic index of plasma, NHDL=Non- High density Lipoproteins

Table 2. Results of control and women on hormonal contraceptives at different age interval

Age interval (Years)	Calcium (mmol/L)	Phos (mmol/L)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	AIP	NHDL-C (mmol/L)	No of women
18 -26 (Control)	2.30±0.46	1.23±0.42	4.51±1.06	0.87±0.40	1.52±0.31	2.68±1.09	-0.29±0.25	2.99±1.07	16
18- 26 (Test)	2.44±0.21	1.07±0.18	4.17±1.06	0.82±0.42	2.10±0.55	1.70±0.74	-0.41±0.23	2.07±0.84	35
P value	0.14	0.06	0.28	0.72	0.03	0.01	0.12	0.01	
Remark	**NS**	**NS**	**NS**	**NS**	**S**	**S**	**NS**	**S**	
27 -35 (Control)	2.57±0.08	1.18±0.16	4.63±1.20	0.75±0.42	1.62±0.48	2.66±1.08	-0.38±0.20	3.00±1.08	19
27- 35 (Test)	2.35±0.29	1.46±0.52	4.37±1.23	1.23±3.34	1.62±0.77	2.44±1.02	-0.42±0.26	2.75±1.00	45
P value	0.01	0.02	0.44	0.52	1.00	0.45	0.51	0.39	
Remark	**S**	**S**	**NS**	**NS**	**NS**	**NS**	**NS**	**NS**	
36 -44 (Control)	2.61±0.09	1.36±0.40	3.67±0.94	0.89±0.57	1.69±0.43	1.58±0.67	-0.36±0.25	1.98±0.66	15
36- 44 (Test)	2.33±0.19	1.11±0.19	4.32±1.14	0.81±0.38	1.47±0.37	2.39±1.01	-0.33±0.28	2.86±1.08	20
P value	0.01	0.02	0.08	0.65	0.11	0.01	0.78	0.01	
Remark	**S**	**S**	**NS**	**NS**	**NS**	**S**	**NS**	**S**	

TC=Total cholesterol, TG=Triglyceride, HDL-C=High density Lipoprotein, LDL-C=Low density Lipoprotein, AIP=Atherogenic index of plasma, NHDL=Non- High density Lipoproteins. Age interval: 18-26-young reproductive age, 27-35-Mid reproductive age and 36-44-late reproductive age. S=significant, NS=Not significant at P=.05

The significant higher level of phosphate seen in women on noristerat (WON) compared to control women (CW), women using oral contraceptives (WOOR), women on Depo Provera (WODP) is in line with the documentation of Moller et al. [24]; Akinloye et al. [25]. They reported significant higher levels of plasma phosphate in women on HCs against women not on contraceptives. However, the significant higher value seen in our work is contrary to the reports of Dante et al. [21]. Dante et al. [21], reported that injectable HCs reduce plasma phosphate level when compared to OCs users. The higher values seen in phosphate in users of noristerat could be as a result of the opposite directional movement of calcium and phosphate in and out of cells. The hypocalcaemic effect seen in noristerat probably stimulated hyperphosphataemic effect. The hypophosphataemic effect is in the order of depo provera>noristarat>oral contraceptives as seen in our work. Generally, the results of calcium and phosphate suggests that injectables (especially depo provera) does not enhance bone mineralization which implies that the use of depo provera injectable for a long time could prone women in developing osteoporosis as a result of bone demineralization.

In addition, when total cholesterol and triglycerides were considered, significant differences were not seen in the various groups of women that used different HCs at *P*=.05. However, higher levels of TC and TG were seen in women using oral contraceptives (WOOR) and noristerats (WON) respectively. Our finding is in line with the reports of World Health Organization [29] on steroid hormones contraceptives. They stated that steroid contraceptives do not affect lipid profile fractions. Again, Okere et al. [2]; Gali et al. [3], stated in their respective studies that oral contraceptives do not affect TC. In addition, Asare et al. [4], reported that oral or HCs does not affect TG levels among users. However, our finding is contrary to the reports of Berenson et al [5]; Naz et al [6]; Dilshad et al. [30]. They all reported in their respective work that HCs caused a significant increase in TC and TG compared to women not on contraceptives. Furthermore, Sufa et al. [31], reported a high TG levels in women using OCs.

Again, significant higher values of HDL-C were seen in women using depo provera (WODP) when compared to Noristerat (WON) and women on contraceptives (WOC). Our finding is contrary to the reports of Asare et al. [4]. They reported that HCs whether oral or injectables did not affect HDL-C lipid fractions. However, our finding of higher level of HDL-C among women using injectables contraceptives (depo provera) compared to control women is in line with the reports of Okere et al. [2]; Dilshad et al. [30]. They reported in their respective studies that the use of injectable contraceptives induces a significant increase in HDL-C plasma level compared to OCs.

In addition, the higher level of LDL-C among women using oral contraceptives seen in our work is in line with the report of Berenson et al [5]; Naz et al. [6]; Sufa et al. [31]. However, it is contrary to the report of Gali et al. [3]. They reported oral or injectables contraceptives had no significant difference in LDL-C values among women using HCs. The significantly lower level of LDL-C among women using Injectable contraceptives observed in our study is contrary to the findings of Adekunle et al. [32]. They reported that the use of Injectable contraceptives (IC) induced higher levels of LDL-C compared against women not on contraceptives. However, the result of lower values of LDL-C seen in women using Injectable contraceptives (IC) in our work is in agreement with the reports of Asare et al. [4]; Dilshad et al. [30]. They reported lower levels of LDL-C among women using Injectable contraceptives (IC) compared to women not using contraceptives.

When atherogenic markers were considered (Table 1), the significant higher values of AIP seen in women on oral contraceptives (WOOR) and women on Noristerat (WON) against women on Depo provera (WODP) are in line with the reports of Okere et al. [2]; Gali et al. [3]. They reported a similar significant increase in atherogenic indices (Castilli index 1 and 2) among women using OCs compared against women on Injectables and women not on contraceptives. Soska et al. [33], also reported that the use OCs induced significant increase in AIP among women using OCs compared to women not on contraceptives. More so, the significant lower values of NHDL-C seen in women on Depo provera (WODP) against control women (CW) as well as the significantly higher values of NHDL-C seen in women on oral contraceptives (WOOR) and women on Noristerat (WON) when compared to women on Depo provera (WODP) are also in agreement with the reports of Berenson et al. [5]. Berenson et al. [5], also reported a significant increase in NHDL-C among women using

OCs. They further reported significant reduction in these atherogenic indices among women using ICs like depo provera compared to control women.

The results of increased lipid parameters (and decreased HDL-C) and atherogenic indices seen among users of OCs in this study suggest that the use of oral contraceptives could be a risk factor for dyslipidaemia viz-a-viz not having cardioprotective effect. OCs has been associated with risk of cardiovascular diseases due to estrogen enhancing lipid metabolism within the liver cells. More so, OCs have also been seen to suppress hepatic lipase by ethinyl estradiol which slows the transport to the liver of HDL-bound cholesterol. This could account for the decreased level of HDL-C seen in the OCs users. The increase in AIP and NHDL-C among these women further indicates that oral contraceptive is probably a risk factor for dylipidaemia and cardiovascular complications among women. However, in those that used injectable contraceptives like noristerat and depo provera, the lipid parameters such as TG and TC were not affected. Higher and lower levels of HDL-C and LDL-C respectively were also seen. Again, atherogenic indices such as AIP and NHDL-C were significant reduced compared to control women and women on oral contraception. The reduction could be as a result of the overall counter effects of the progestin-only injectable contraceptives (depoprovera and noristerat). Though, it was also observed in this study that Depo provera was more cardio-protective compared to noristerat because Depo Provera tend to have significantly lower level of AIP and NHDL as well as higher level of HDL-C compared to Noristerat. This observation could be that though noristerat (norethisterone) is a progestin-only injectable contraceptives but it is containing minute fractions of estrogen since it is a synthetic derivative of estradiol (estradiol methyl ether). The results suggest that depo provera (midroxyprogesterone) injectable contraceptives confer more beneficial and do not pose cardiovascular risks compared to noristarat and combined oral contraceptives. The observation could be as a result of the fact that depo provera (midroxyprogesterone) is a progestin-only contraceptive that does not contain estrogen unlike noristerat thus exerting a significant increase and decrease levels of HDL-C and LDL-C respectively.

The significant lower values of LDL-C and NHDL as well as the significant higher values of HDL-C were seen in young reproductive women using contraceptives within the age group of 18 – 26 years indicate lower or no risk of cardiovascular dysfunction or dyslipidaemia. Mineralization of the bones was also not affected due to non-significant levels of calcium and phosphate. More so, the significant lower value of calcium in the plasma at age interval of 27-35 years also indicate that women within this age group are less prone to bone demineralization as a result of movement of calcium from the plasma into the bones. Furthermore, the significant higher values of LDL-C and NHDL-C at age interval of 36-44 years suggest risk of cardiovascular dysfunction or dyslipidaemia. Higher values NHDL-C and atherogenic lipid parameters such as LDL-C are strongly associated development of cardiovascular diseases. Finally, the significant lower values of calcium and phosphate also suggest bone mineralization. The movement of minerals especially calcium from plasma into bone has been indicated to enhance bone mineralization thereby preventing the development of osteoporosis as seen mostly in post-menupausal women.

5. CONCLUSION

The results obtained demonstrated that injectable contraceptives exerted more beneficial effect on lipid metabolism than oral contraceptives. However, oral contraceptives had more bone mineralisation effect compared to women on injectables contraceptives. When age interval were considered, young women at age 18-26 had low risk of dyslipidaemia and cardiovascular diseases compared to age 27-35 years while age 36-44 years but were less prone to bone demineralisation viz-a-viz development of osteoporosis.

6. RECCOMMEDATION AND FUTURE WORK

It is therefore recommended that plasma minerals such as calcium, phosphate, magnesium as well as lipids, and atherogenic indices of women should be determined on routine basis before hormonal contraceptives are administered or recommended. For future work, larger number of participants will be recruited, factors such as sedentary lifestyle, type of food regularly consumed, egree of exercise, domestic and work pressure, family history, and the presence of other diseases such as some cancers (e.g. breast and colon) and asthma will also be considered.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

Informed consent was obtained from all subjects before recruited upon approval by the Department of Medical Laboratory Science, Rivers State University, Port Harcourt

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Available:https://doi.org/10.1371/journal.pone.0197922

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/58744