INTERNATIONAL JOURNAL OF NATURAL AND APPLIED SCIENCES (IJNAS), VOL. 3, NOS.1& 2 (2008); P. 119 – 122, 2 TABLES.

Improved atherogenic risk predictor indices associated with lower oestrogen/progestin oral contraceptive formulation

H. U. Nwanjo[*1] and A. U.Opara[1]

ABSTRACT

We studied 30 women taking oral contraceptive and 40 women taking no hormones, to determine the relation of plasma lipoproteins concentrations and atherogenic risk predictor indices to low oestrogen/progestin oral contraceptive (OCs) formulation. Premenopausal women, using oral contraceptive containing a relatively low dose of oestrogen and low dose of progestin (Duofem cycle) had a significantly increased total cholesterol (5.22 ± 0.13 mmol/L) and high density lipoprotein cholesterol (1.73 ± 0.09 mmol/L) ($p<0.05$) than did those not using hormones (4.63 ± 0.12. mmol/L) and (1.25 ± 0.06 mmol/L) for total cholesterol and high density lipoprotein cholesterol respectively. Also the mean atherogenic risk predictor indices; HDL-C/TC (0.33 ± 0.02) increased and LDL-C/HDL-C (1.67 ± 0.27) decreased in contraceptive users when compared to non-users (0.27 ± 0.02) and (2.26 ± 0.22) for HDL-C/TC and LDL-C/HDL-C respectively showing that these hormone formulations are non-atherogenic and desirable. These findings showed that these OC formulations appear to balance contraceptive efficacy/safety with tolerability.

INTRODUCTION

Oral contraceptives (OCs) have been an effective mainstay of hormonal contraception since they were introduced 40 years ago. They are the single most commonly used and requested method of reversible contraceptive, with a perfect-use failure rate of less than 1%. Many improvements and changes in oral contraceptive formulations have occurred over the past four decades, most notably, the significant reduction in both oestrogens and progestin doses to enhance the safety profile. More recent advances include the introduction of three new progestins (norgestimate, desogestrol, and drospirenone) and new oral contraceptive regimens utilizing even lower oestrogens doses-the most recent ethinyl oestradiol (EE): These oral contraceptive formulations aim to balance contraceptive efficacy/safety with tolerability.

Despite all these improvements, controversy exists regarding the adverse and beneficial effects of oral contraceptive use. The influence of OCs on lipoprotein metabolism is dependent on the characteristics and dose of both progestin and oestrogen components (Wallace *et al*, 1979). The progestin component of OCs can alter the level of lipids (such as cholesterol) in the blood. Although oestrogen works against this effect by increasing beneficial high-density lipoprotein (HDL) and lowering harmful low density lipoproteins (LDL), the progestin component opposes the oestrogen and does the opposite. Because high levels of LDL and depressed levels of HDL can cause fatty plaque to build up in the arteries, these lipoprotein changes are considered as independent factors for coronary heart diseases (Miller and Miller, 1975).

Evidently, lipoproteins, which are central to the metabolism of the body, have become increasingly important in clinical practice primarily due to its association with coronary heart disease (CHD). Many national and international epidemiological studies have demonstrated that there is a clear association with the development of atherosclerosis, angina pectoris, coronary thrombosis and diabetic ketosis, which are mainly caused by abnormal lipid metabolism (Okolo, 1988).

However, in America and European countries (with a high rate of CHD), abnormal lipoprotein metabolism has been implicated in the higher incidence of CHD in OC users when compared with nonusers (Stadel, 1981). There is scanty information regarding the effect of contraceptives especially newer fomulations on coronary heart diseases in Nigerian women (which is quite different from developed countries pattern of serum lipoproteins). This present study is an endeavour to investigate the effect of a newer contraceptive on lipoproteins and atherogenic risk predictor indices within our environment (Owerri, Imo State).

MATERIALS AND METHODS

Subjects

Thirty (30) healthy women, aged 16-35 years who were of proven fertility who have been on oral contraceptives for a period of time and were attending family planning clinic at Federal Medical Centre, Owerri for follow ups were studied. These subjects were using duofem cycle, a triphasic oral contraceptive containing 28 pills. Each white tablet contains 0.3mg Norgestrel and 0.03mg ethinyl estradiol and each brown tablet contains 75mg ferrous fumarate.

[*]Corresponding author. Email:

Manuscript received by the Editor July 19, 2005; revised manuscript accepted August 29, 2007.
[1]Department of Medical Laboratory Science, Imo State University, Owerri , Nigeria.

Another 40 healthy women within the same age range who have never used any form of contraceptive, and were attending the family planning clinic for the first time served as control subjects. Subjects excluded from this study included pregnant women, lactating mothers, women with unexplained abnormal vaginal discharge. Also excluded were those with active liver disease, over 35 years old and smoke, have history of high blood pressure, heart disease or stroke, clotting problems, diabetes, and women who know or suspect that they have cancer of the breast. The purpose and detailed procedure were well explained to all the subjects and their written consent were obtained prior to the study as recommended by WHO (TDR, 2001 and TDR, 2002).

Laboratory assays

Using 10ml syringe, 8ml of fasting blood sample was taken from each subject by venepuncture. The blood collected was immediately transferred into Na_2EDTA bottles. The anticoagulated blood was then centrifuged at 3000 rpm using Wisperfuge model 1384 centrifuge (Tamson, Holland) for 10min to facilitate separation. The plasma obtained after centrifugation was used for plasma lipids. Total cholesterol was measured using the method of Zak (1957), in which the colour intensity in acetic acid with ferric chloride and sulphuric acid gives the amount of cholesterol present. Triglyceride was measured using the extraction method of Mendez *et al*, (1975), while HDL-cholesterol was measured using the method of Lopez-Vitrella *et al*, (1977). LDL-cholesterol and VLDL trighyceride values were calculated by a modification of the Friedewald formular (Sandkamp 1990). The atherogenic risk predictor indices were calculated using the formular of Dobiasova and Frohlich (2001).

Statistical analysis

All values were expressed as mean ± SD. The statistical analysis was carried out using students' t-test to detect differences in the concentration of tests and control. Tests with a probability value < 0.05 were considered statistically significant.

RESULTS

The results of the mean ages, body weights and blood pressures in all the subjects are shown in table 1. The values for the age, body weight and systolic and diastolic blood pressures were not significantly different from the control levels.

Table 1. Mean value of ages, body weight and blood pressures in all the subjects (mean ± S.D.).

Parameters	Contraceptive (test)	Control
Age (yrs)	25.0 ± 2.1	24.0 ± 1.6
Body weight (kg)	58.2 ± 2.6	54.6 ± 2.0
Systolic Bp (mmHg)	106.06 ± 3.46	104.42 ± 2.71
Diastolic Bp (mmHg)	69.18 ± 2.79	66.46 ± 1.96

None was significant.

Table 2 shows the mean values of plasma lipoprotein levels in all the subjects. The mean values of plasma total cholesterol and HDL-cholesterol significantly increased in contraceptive users ($p<0.05$) when compared with the value in non-users. There was a positive correlation between the duration of use of this drug and the plasma concentration of total cholesterol ($r = 0.6645$) and HDL-cholesterol ($r = 5264$). There was no significant difference of LDL-cholesterol and triglycerides in contraceptive user when compared with non-users. Also shown in Table 2 are the mean ratios of the atherogenic risk predictor indices HDL–C/TC and LDL-C/HDL-C in contraceptive users and non-users. The mean HDL-C/TC ratio increased and LDL-C/HDL-C ratio decreased in contraceptive users when compared with non-users.

Table 2. Mean values of plasma lipids and atherogenic risk predictor indices

Parameters	Contraceptive (test)	Control
Total cholesterol (mmol/L)	5.22 ± 0.13*	4.63 ± 0.12
HDL-cholesterol (mmol/L)	1.73 ± 0.09*	1.25 ± 0.06
LDL-cholesterol (mmol/L)	2.93 ± 0.11	2.83 ± 0.09
Triglycerides (mmol/L)	1.29 ± 0.11	1.20 ± 0.09
HDL-C/TC	0.33 ± 0.02*	0.27 ± 0.02
LDL-C/HDL-C	1.67 ± 0.27*	2.26 ± 0.22

* Significantly different from control value ($p<0.05$).

DISCUSSION

In this study, it was shown that oral contraceptive of low oestrogen/progestin formulation have a significant effect on the plasma lipid profile as well as on atherogenic risk predictor indices in women.

The findings of this present investigation showed no significant difference in both systolic and diastolic blood pressure readings in women using OCs of low oestrogen/progestin formulation when compared with controls which is consistent with other work (Wallace *et al*, 1979). It is inconsistent with the work of Agbedana *et al*, (1988).

Also in this study, use of OCs of low oestrogen/progestin formulation resulted in significantly high values for plasma total cholesterol ($p<0.05$), which is consistent with those of Bradley *et al*, (1978) and Agbedana *et al* (1988) who reported high elevation of total cholesterol by low oestrogen containing pills. There was no significant increase of LDL-cholesterol ($P<0.05$) in OCs users while HDL-cholesterol significantly increased when compared to non-users. This is in consonance with the work of Rossner *et al*, (1971) and Taylor *et al* (1982). The triglycerides remained unchanged in women taking OCs. Knopps *et al* (1982) suggested that the relative oestrogen and progestin potency as well as the type of formulation are important determinants of lipid profiles in women using OCs.

In this present study we also looked at the atherogenic risk predictor indices: LDL-C/HDL-C and HDL-C/TC in these women using OCs of low oestrogen/progestin formulation. The atherogenic risk predictor index (HDL-C/TC) significantly increased to 0.33 ± 0.02 ($p<0.05$) which is within the reference range for HDL-C/TC ratio of ≥ 0.3 (in our environment) and LDL-C/HDL-C decreased significantly ($p<0.05$) to 1.67 ± 0.27, which is also within the reference range for LDL-C/HDL-C of ≤ 2.3 (in our environment) in women using OCs when compared with non users. This shows that these ratios for the women using OCs are desirable and antiatherogenic. This implies that taking of OCs with new formulations of new progestins (norgestrel, (0.3mg) and even lower oestrogen doses – ethinyl estradio (0.03mg) will reduce the risk of developing atherosclerosis as LDL-cholesterol level will be lowered and HDL-cholesterol increased. The increase in HDL-cholesterol is effective in the prevention of atherosclerosis as it is related to the transport of extra cholesterol from the organs to the liver for it to be either reused or eliminated (Agarbaek *et al*, 1995).

High concentration of total cholesterol, LDL-cholesterol, high blood pressure and low level of HDL-cholesterol concentration have been directly and independently associated with increase incidence of CHD while the reverse reduces the incidence of cardiovascular disease (Kawase *et al*, 2000). Modification of the OC formulations which have occurred over the past decades, most notably, the significantly reduction in both oestrogen and progestin doses is one way that serum cholesterol may be reduced in women using OCs.

The mechanism of oestrogen and progesterone effect on plasma lipoprotein metabolism has not been extensively investigated. The increased HDL may be due to increased liver synthesis or production from increased hydrolysis of triglyceride-rich particles by the enzyme lipoprotein lipase (Usman and Hosno, 2000).

CONCLUSION

Since their introduction more than 40 years ago, OCs have been a mainstay of hormonal contraception. The risk of serious health concerns such as atherosclerosis or coronary heart diseases, associated with the high oestrogen and progesterone doses of the original

formulations led to the introduction of new, less androgenic progestins and to significant reductions in hormone doses.

Nearly all OC formulation currently available in Nigeria contain ethinyl estradiol 20-35µg and newer progestins, including norgestimate, desogestrel, norgestrel and drospirenone and provide users with the same excellent contraceptive efficacy as did the higher-dose regimens albeit with improved atherogenic risk predictor indices and safety profiles. This study has shown that these OC formulations appear to balance contraceptive efficacy/safety with tolerability.

REFERENCES

Agarbaek, M., Gerdes, L. U. and Richelsen, B. (1995). Hypocholesterolaemic effect of a new fermented milk product in healthy middle aged men. *Eur. J. Clin. Nutr. 49*: 346-352.

Agbedana, E. O., Iwuafor, C. and Otolorin, E. O. (1988). Changes in plasma lipids and lipoproteins in oral contraceptive users. *Proc. 1st Afric. Conf. on Biochem. of lipids. 1*: 165-174.

Bradley, D. D., Wingerd, J., Petitti, D.B., Krauss, R.M. and Ramcharan, S. (1978). Serum high density lipoprotein cholesterol in women using oral contraceptives. *New Engl. J. Med. 299:* 17-23.

Kawase, M., Hashimoto, H., Hosoda, M., Morita, H., and Hosono, A. (2000). Effect of administration of fermented milk containing whey protein concentrate to rats and healthy men on serum lipids and blood pressure. *J. Dairy Sc. 83*: 255-263.

Knopp, R.H., Walden, C.E., Wahl, P.W. and Hoover, J.J. (1982). Effects of oral contraceptives on lipoprotein triglyceride and cholesterol. Relationships to estrogen and progestin potency. *Am. J. Obstet. And Gynae. 142*:725-732.

Lopez-Villrella, M.F., Stone, P., Ellis, S. and Coltwell, J.A. (1977). Cholesterol determination in high density lipoprotein, separated by three different methods. *Clin Chem. 23*: 882-884.

Mendez, E., Franklein, J. and Sahegan, B.H. (1975). Simple manual method for determination of serum triglycerides. *Chemistry. 21:* 760-770.

Miller, G. J. and Miller, N.E. (1975). Plasma high density lipoproteins concentration and development of ischaemic heart disease. *Lancet. 1*:19-25.

Okolo, F. (1988). Dietary lipids: nutritional and epidemiological factors involved in the disease resulting from their metabolism. *Proc. 1st Afric. Conf. on Biochem. of lipids. 1*: 92-102.

Rossner, S., Larison-Cohn, Y., Carlson, L.A. and Boberg, J. (1971). Effect of an oral contraceptive agent on plasma lipids, plasma lipoproteins, the intravenous fat tolerance and the post-heparin lipoprotein lipase activity. *Acta. Med. Scand. 190:* 301-312.

Sandkamp, M., Funke, H., Schulte, J., Kahlar, E. and Assmann, G. (1990). Lipoprotein (a) is an independent risk factor for myocardial infarction at a young age. *Clinical Chemistry. 36*: 20 – 23.

Stadel, B.V. (1981). Oral contraceptives and cardiovascular disease. *New Engl. J. Med.* 612 : 981-988.

Taylor, G.O., Agbedana, E.O. and Ogo, O.A. (1982). Plasma high-density lipoprotein cholesterol levels during long-term use of an oral contraceptive in Nigerian women. *Br. J. Obstet Gynae. 89*: 944-952.

TDR (2001). Handbook of Good laboratory practice (GLP). Quality Practices for regulated non-clinical research and development. *TDR/PRG/GLP/01.2.*

TDR (2002). Workbook for investigators. UNDP/World bank/WHO special programme for research and Training in Tropical Diseases. *(TDR)/PRD/GCP/02.1b).*

Usman, H. and Hosono, A. (2000). Effect of Administration of *lactobacillus gasseri* on serum lipids and faecal steroids in hypercholesterolaemic rats. *J. Dairy Sc. 83:* 1705-1711.

Wallace, R.B., Hoover, J., Barrett-Connor, E., Rifkind, B.M., Hunning lake, D.B., Mackenthum, A. and Heis, G. (1929). Altered plasma lipid and lipoproteins levels associated with oral contraceptive and oestrogen use. *Lancet 2*: 3-6.

Zak, B. (1957). Determination of total, free and esterified cholesterol using reaction with ferric chloride and sulphuric acid. *Am. J. Clin. Pathol. 27*: 583-589.