Original Article

NON-ENZYMATIC ANTIOXIDANT STATUS OF WOMEN USING FOUR DIFFERENT METHODS OF CONTRACEPTION

Akinloye O[1], Oyabiyi SA[2], Oguntibeju OO[3], Arowojolu AO[4]

ABSTRACT

Objective: To investigate antioxidant status of women on four different methods of contraception.

Methodology: Sixty non-pregnant women aged 16-45 years on oral contraceptive pills, injectables, Norplant and intra-uterine contraceptive devices (IUD) attending the Family Planning Clinics of the University College Hospital (UCH) and Adeoyo Maternity Hospital, Ibadan were recruited for the study. Fifty-eight apparently healthy women aged 16-45 years who were not on any contraceptive served as a control group. The body mass index (BMI) of all participants (subjects and controls) was determined following standard protocol. Serum levels of ascorbic acid, tocopherol, malondialdehyde, bilirubin, creatinine, uric acid, total protein and albumin were determined using standard spectrophotometric methods. Progesterone was estimated by the chemilumiscence method while selenium was determined by atomic absorption spectrophotometry (AAS).

Results: The BMI was significant in women on oral contraceptive pills (OCP) when compared to the control group ($P<0.05$) but insignificant ($P>0.05$) in intra-uterine device (IUD), injectables and Norplant users. The mean serum ascorbic acid ($P<0.01$), tocopherol ($P<0.05$), total protein ($P<0.01$), albumin ($P<0.05$), uric acid ($P<0.05$), selenium ($P<0.01$) of women on OCP were significantly lower when compared to the control group but insignificant ($P>0.05$) in users of other contraceptive methods. Serum levels of malondialdehyde was significantly elevated in women on OCP ($P<0.01$) than in control group and insignificant $P>0.05$) in users of other contraceptive methods. There was no significant association between progesterone and antioxidants in women on OCP, IUD, injectables and Norplant.

Conclusion: Oral contraceptive pills showed a significant decreasing effect on the antioxidant status of its users while IUD, injectables and Norplant did not indicate any significant effect. Routine monitoring of the antioxidant status of women on different methods of contraceptive particularly those on OCP is recommended.

KEY WORDS: Non-enzymatic; Antioxidants; Women; Contraceptives.

Pak J Med Sci April - June 2010 Vol. 26 No. 2 374-379

How to cite this article:

Akinloye O, Oyabiyi SA, Oguntibeju OO, Arowojolu AO. Non-enzymatic antioxidant status of women using four different methods of contraception. Pak J Med Sci 2010;26(2):374-379.

Correspondence:

Dr. O.O. Oguntibeju
Dept. of Biomedical Sciences, Faculty of Health & Wellness Sciences,Cape Peninsula University of Technology, Bellville Campus 7535, South Africa.
Email: oguntibejuo@cput.ac.za

* Received for Publication: September 19, 2009
* Revision Received: January 26, 2010
* Revision Accepted: February 1, 2010

INTRODUCTION

Oxidative stress is associated with the development of various disease conditions such as cardiovascular disease and cancer.[1,2] Among conditions known to influence oxidative stress, the use of oral contraceptives in women has been a matter of concern.[3] Research studies have

dealt with the influence of oral contraceptives use on changes in carbohydrate, lipid, enzymes, mineral and vitamin metabolism since their introduction in the prevention of pregnancy.[4] Data on the relationship between oxidative stress and oral contraceptives are scarce and still remain a subject of debate. It has been suggested that (although not widely accepted) that estrogens have an antioxidant effect that may contribute to its protective effects on the cardiovascular system through inhibition of lipid oxidation.[5] In vitro studies by Saha et al. and Chiang et al. reported that estrogens significantly reduce the oxidative damage to lipids exposed to free radical generating systems.[6,7] Few in vivo studies noted an antioxidant effect of estrogen on rats and women receiving hormone replacement therapy (HRT).[8] However, only a few studies have examined the relationship between combined oral contraceptives containing estrogens and progesterone and oxidative status in developing countries such as Nigeria.

Studies by Kose et al.; Sissan et al. demonstrated significant increase in blood lipid peroxides with a corresponding decrease in antioxidant status.[9,10] The aim of the present study was to assess antioxidant status and biomarkers of oxidative damage of lipids of women on oral contraceptive pills, injectables, Norplant and intrauterine device.

METHODOLOGY

Study Population: The study population was non-lactating, contraceptive women and non-contraceptive non-lactating women recruited from two hospitals in Ibadan, Western Nigeria.

Study Design: This was a cross-sectional randomized study designed to investigate the effects of contraception on antioxidant status of women.

Selection of Subjects: In this study, 60 non-lactating women of reproductive age 16-45 years (mean age 33.4 + 0.9) on one of the four selected methods of contraception and 58 apparently healthy control subjects (non-lactating, non-contraceptive users) of same age group recruited from the University College Hospital (UCH) and Adeoyo Maternity Hospital, Ibadan were investigated. The women on contraceptives were categorized into: oral contraceptive (n=10), injectables (n=10), intrauterine device (n=31) and Norplant implant (n=9). The mean duration of exposure to contraceptive was (7.0 + 1.8) years. A questionnaire was administered to obtain relevant demographic information from the participants.

Ethical Consideration: Ethical approval was obtained from the Ethical Review Committee, Ministry of Health, Ibadan, Oyo state and all the participants gave informed consent before participation in the study.

Exclusion Criteria: These include pregnant, lactating, smoking and alcoholic women. Women with acute or chronic illnesses or taking any other medications that could potentially affect antioxidant status were also excluded.

Measurement of Height: Measurement of height was taken in standing position using a stadiometer. The height was measured to the nearest 0.01m. When measuring height, the subjects and controls stood straight with the head positioned such that the Frankfurt plane was horizontal, feet together, knees straight and heels, buttocks and shoulder blades in contact with the vertical surface of the wall, arms hanging freely at the sides with the palm facing the thighs.

Measurement of Weight: A beam balance with non-detachable weights was used. The body weight was recorded to the nearest kg. The balance situated on a hard flat surface and checked for zero balance before each measurement. The subject stood unassisted in the center of the platform and asked to look straight ahead, standing relaxed and in light clothing. The BMI was calculated from the average height and weight (Weight/Height) in square metres.

Sample Collection: Blood samples were collected via vene-puncture, avoiding prolonged venous stasis from both the subjects and the controls. Ten (10) mls of blood was collected from each of the participants into lithium heparinized anti coagulated tube and quickly kept in a dark polythene bag. The samples were spun using MSE centrifuge (Centaur 2) at 3,000 rpm for 5 minutes and the plasma stored frozen at -20^{0}C

and analysis was performed within 72 hours of blood collection.

Analytical Methods: A Simple colorimetric method previously described by Aye Kyaw was used to determine ascorbic acid concentration.[11] Tocopherol was measured using the method described by Baker and Frank at 520 nm. Biuret method was used in the determination of total protein at an absorbance of 550 nm.[12] While albumin level was determined using Bromocresol Green method and absorbance measured at 630 nm. The uricase colorimetry (enzymatic method-YD uric acid kit) was used for the determination of uric acid concentration. Bilirubin was determined colorimetrically. Creatinine was determined by Jaffe colorimetric method. Lipid peroxidation was assessed by measuring thiobarbituric acid reactive substances (TBARS) and the results expressed as ìmol/L. Selenium was measured using atomic absorption spectrophotometric technique (AAS). Progesterone was determined by competitive immunoassay technique using Luminescence Reaction on Vitros ECiQ Immunodiagnostic System.

Statistical Analysis: SPSS (Windows Version 10.0) was used for statistical analysis. The findings were expressed as the mean ± SEM. Statistical analyses were undertaken using the student-t test and Pearson's rank correlation test. Comparison of mean between and within all groups was done using multiple analysis of variance (ANOVA). A p value <0.05 was set as significant.

Internal Quality Control: Serial analyses of standards were done and the mean and standard deviation were calculated. During each run, these standards were included as controls. Values that fell within ±2SD were accepted and the results of the participants reported.

RESULTS

Admission Characteristics of the Subjects: More women between the ages 28-33 years were on the four different methods of contraception, followed by those of 40-45 years and the least number of women were found among the 46-50 years old. It was also observed that more women between the ages of 26-30 were on OCP and injectables whereas, more women between the 31-35 age were on IUD and women on Norplant were between 36-40 years old.

There was no significant difference (>0.05) b etween the BMI and blood pressure of women on IUD, injectables and Norplant when compared to the control group. As shown in Table 1, there was a significant difference between BMI and systolic blood pressure of women on OCP when compared with the control group. As represented in Figure 1, 16.7% of the women were on OCP, 15% on Norplant, 16.7% on injectables and 51.7% on IUD.

We observed significant reduction in the mean serum levels of malondialdehyde of women on intrauterine device (IUD) when compared to the controls (P<0.05). Serum ascorbic acid level was positively associated with albumin (r=0.782, P<0.01). A significant positive association between serum tocopherol and creatinine (r=0.627, P<0.05) was also observed. We noted that serum uric acid was positively correlated with albumin (r=0.662, P<0.05) and selenium (r=0.816, P<0.01). However, serum MDA was negatively correlated with uric acid (r=-0.883, P<0.05).

There was no significant association between serum progesterone and other biochemical parameters of women on injectables. Serum uric acid concentration was however positively

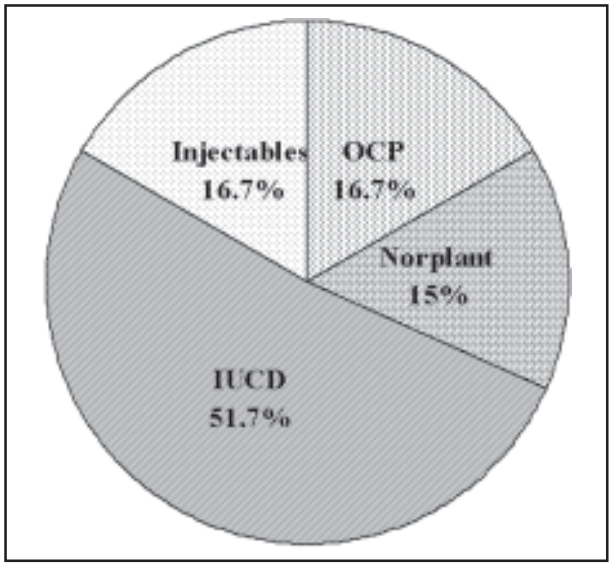

Figure-1: Percentage distribution of women on different methods of contraception.

Table-I: Anthropometric and blood pressure characteristics of women on OCP and controls

Variables	*OCP* $\overline{X} \pm SEM$	*Control* $\overline{X} \pm SEM$	*t-value*	*P-value*
Weight (kg)	55.8 ± 1.5	57.7 ± 2.1	0.74	NS
Height (m)	1.49 ± 0.02	1.60 ± 0.03	3.23	P<0.05
BMI (kg/m2)	25.4 ± 0.7	22.7 ± 1.0	2.51	P<0.05
Systolic BP (mmHg)	126 ± 5	105 ± 2	3.88	P<0.05
Diastolic BP (mmHg)	81 ± 4	73 ± 2	1.74	NS

associated with MDA (r=0.688, P<0.05) and albumin (r=0.766, P<0.01).

There was no significant correlation between serum progesterone and other biochemical parameters of women on Norplant. However, a significant negative correlation was observed between serum ascorbic acid and selenium (r=-0.670, P<0.01). Also, uric acid was positively associated with MDA (r=0.674, P<0.01) and albumin (r=0.687, P<0.01).

For women on IUD, injectables and Norplant, the levels of ascorbic acid, tocopherol, selenium, uric acid, creatinine, albumin and total protein did not differ significantly from that of the control group.

Table-II shows that the mean serum concentrations of ascorbic acid (P<0.01), tocopherol (P<0.05), albumin (P<0.05), uric acid (P<0.05), total protein and selenium (P<0.01) in test subjects on OCP were significantly reduced when compared with the corresponding mean values in the controls. The MDA serum levels were significantly higher in women on OCP when compared with the control group.

DISCUSSION

We observed that women between the ages of 16 years to 20 years, who were on either OCP or Norplant, are predominantly single ladies who want to prevent pregnancy as a result of their indulgence in casual sex whereas, those between the ages of 21 years to 25 years, who were on IUD are young ladies who are developing a career or are in school and do not want to fall pregnant, hence the need for a fairly long acting, reversible and effective method of contraception. It was also observed that, of all the methods investigated, more women were on IUD in this study area and they fall between 31 years to 35years of age. The percentage distribution of women on IUD (Figure.1) is in agreement with the study carried out on the distribution of contraceptive use among women of reproductive age in a Turkish population which concluded that IUD was commonly used, with 55% of the 1046 women studied.[4]

Anthropometric indices of women on OCP revealed a slight but insignificant weight loss when compared with the control subjects. This is in contrast with the opinion held that due to the oestrogenic (steroid) effect of combined OCP there may be significant weight gain which could potentially constitute a risk factor for cardiovascular disease. In the absence of disease promoting weight loss, the finding that women on combined OCP did not gain weight as expected may be beneficial.[13]

Table-II: Serum progesterone level and non-enzymatic antioxidant status of women on OCP and controls

Variables	*Patients* $\overline{X} \pm SEM$	*Control* $\overline{X} \pm SEM$	*T-test*	*P-value*
Ascorbic acid (mg/dl	0.46 ± 0.03	0.92 ± 0.09	4.76	P<0.01
Tocopherol (mg/dl)	9.27 ± 0.71	12.27± 0.72	2.97	P<0.05
Bilirubin (mg/dl)	0.29 ± 0.03	0.35 ± 0.04	1.18	NS
Creatinine (mg/dl)	0.36 ± 0.04	0.41 ± 0.05	0.81	NS
Albumin (g/dl)	2.67 ± 0.19	3.33 ± 0.16	2.63	P<0.05
Uric acid (mg/dl)	2.17 ± 0.25	3.16 ± 0.22	3.03	P<0.05
Selenium µmol/L	0.47 ±0.12	1.51 ±0.26	3.62	P<0.05
Total protein g/dl	6.05±0.19	7.15±0.22	3.75	P<0,01
Malondialdehyde (µmol/L)	12.42±1.18	4.67±0.84	5.33	P<0.01
Progesterone (nmol/L)	3.35±1.88	5.56±2.21	0.76	NS

Among conditions known to affect oxidative stress, the use of contraceptives in women has been a matter of ongoing academic argument and discussion. It is known that 50 to 100 million women globally use this method of contraception, however data on the relationship between contraceptives and oxidative are scarce and in some cases conflicting. Free radical production occurs as a consequence of the endogenous reactions and plays an important role in normal cellular functions. Exogenous factors such as drugs, contraceptives have been implicated in promoting radical formation and antioxidant depletion.[14] Antioxidant defense system is a complex and diverse system, and measurement of blood antioxidants may serve as early biomarkers to assess excessive production of free radicals since it is known that oxidative stress may occur when the balance between reactive oxygen species production and antioxidant defense is disrupted.[10,15]

We report that tocopherol-a free radical scavenging antioxidant, acting at the lipid phase (cell membrane) was significantly reduced in subjects on oral contraceptives when compared to the control. In the same vein, ascorbic acid, uric acid and albumin, acting at the aqueous phase (cell's cytosol) were significantly reduced. Also, selenium (an antioxidant trace element) acting at the active site of glutathione peroxidase for possible scavenging process was significantly reduced among the OCP users. The reduction in total protein observed among the OCP users, could be explained by the hypoalbuminaemia seen in these group of women which gives an indication of their nutritional status.

One of the main findings of this study is the significant increase in lipid peroxides as evident by an increased MDA level observed in women on OCP. Studies in rats and humans (women) have reported an increased level of plasma lipid peroxides due to the use of oral contraceptives containing estrogen.[16] Ciavatti et al. in animal studies noted that the elevation of lipid peroxides in OC-treated rats was able to increase the platelets aggregation exposed to thrombin.[17] Such an effect that is potentially involved in the development of venous thrombo-embolism may be blocked by antioxidants as tocopherol.[18] Unfortunately, the concentration of tocopherol and other important antioxidants such as ascorbic acid and selenium were significantly reduced in women on OCP in our study. The question is whether such an increased level of lipid peroxides could have an impact on the health of OCP users. It should however be noted that oxidative damage to lipids and lipoproteins is a process potentially involved in the development of atherosclerosis. Our findings also corroborate that of Saha et al.[6] which showed that OCP use caused significant extent of lipid peroxidation with consequent reduction in the levels of antioxidants such as asrcobic acid and tocopherol and increased MDA level.

In this study, there was no significant difference in BMI and other biochemical parameters of women on injectables, IUD and Norplant when compared with control subjects.

Among the women on OCP, serum ascorbic acid was positively associated with albumin. There was also a significant positive association between the serum tocopherol and creatinine while serum uric acid was positively correlated with albumin and selenium. These findings suggest that ascorbic acid and uric acid, both water soluble antioxidants acting in the cell cytosol to scavenge reactive oxygen species and albumin, a transport protein may have a synergistic role and albumin may play a role in their excretion. However, the association observed between the serum tocopherol and creatinine remains unclear. Also, selenium, a micronutrient at the active site of glutathione peroxidase, may act synergistically at the cell cytosol playing a contributory role in scavenging reactive oxygen species. Among the women on injectables, serum uric acid concentration was positively associated with MDA and albumin. This finding may suggest that uric acid may play a contributing role in the scavenging process of hydroperoxides.

Limitations of the Study: This study was limited by the sample size. Because of logistic and time constraint, the authors could not recruit more participants into the study as would have wanted. Cost also played an

important role in the number of participants recruited. The authors did not secure any financial support from the institutions hence the cost of doing the work was borne by the authors. The inability to assess the dietary intake of participants and investigate its possible correlation with the measured parameters and oxidative or anti-oxidative status is also a limitation. However, in our opinion, these limitations did not significantly affect the objectives of the study and its contribution to the pool of knowledge in the field.

CONCLUSION

The association between contraceptives and oxidative stress remain a matter of concern and academic debate. Estrogens are recognized to be beneficial in the prevention of atherosclerosis although they are capable of inducing oxidative stress which is involved in the development of atherosclerosis. The results of this study showed that OCP is associated with a significantly altered oxidative status in women on OCP while IUD, injectables and norplant showed no significant effect. The increase in MDA levels and decrease in selenium, ascorbic acid and tocopherol strongly support the hypothesis. Further research is suggested to elucidate its real impact as a cardiovascular risk factor in OCP users and routine monitoring of the antioxidant status of women on different methods of contraception may be beneficiary.

REFERENCES

1. Haliwell B. Free radicals and antioxidants: A personal view. Nutr Rev 1994;52:253-266.
2. Knight JA. Review: Free radicals, antioxidants and the immune system. Ann Clin Lab Sci 2000; 30: 145-158.
3. Pincemail J, Vanbelle S, Gaspard U, Collette G, Haleng J, Cheramy-Bien JP *et al*. Effect of different contraceptive methods on the oxidative stress status in women aged 40-48 years from the ELAN study in the province of Liege, Belgium. Hum Reprod 2007;10:1-9.
4. Bozkurt N, Ozkan S, Onan A, Korucuoglu U, Aygun R, Himmeloglu O. Distribution of contraceptive use in a Turkish population. Eur J Obstet Gynaecol Reprod Biol 2006:131(1): 52-55.
5. Ling S, Komesaroff P, Sudhir K. Cellular mechanisms underlying the cardiovascular actions of estrogens. Clin Sc 2006;111:107-118.
6. Saha A, de AU, Sengupta C. Ethinyl estradiol; its interaction on blood-lipid. Ind J Exp Biol 2000;38:906-911.
7. Chiang K, Parthasarathy S, Santanam N. Estrogen, nuetrophils and oxidation. Life Sc 2004;75:2425-2428.
8. Misra R, Mangi S, Joshi S, Mittal S, Gupta SK, Pandey RM. Lycored as an alternative to hormone replacement in lowering serum lipids and oxidative stress markers: A randomized controlled clinical trial. J Obstet Gynaecol Res 2006;32:299-304.
9. Kose K, Dogan P, Ozesmi C. Contraceptive steroids increase erythrocyte lipid peroxidation in female rats. Contraception 1993;47:421-425.
10. Sissan MA, Menon VP, Leelamma S. Effects of low-dose oral contraceptive oestrogen and progestin on lipid peroxidation rats. J Int Med Res 1995;23:272-278
11. Aye Kyaw. A simple colorimetric method for ascorbic acid determination in blood plasma. Clin Chim Acta 1977;86:153-157.
12. Baker H, Frank O. Clinical vitaminology methods and interpretation. John Willey & Sons. NY, USA:1968.
13. Mishell DR. Oral steroid contraception: In infertility contraception and reproduction endocrinology. 3rd Edition Boston MA Blakwell Sc Publ 1991:839-847.
14. Kelly J, Frank TJ. Use of antioxidants in the prevention and treatment of disease J Int Fed Clin Chem 1998;10:21-32.
15. Khanghani S, Pasalar P, Adibi B, Qaraati M, Bagherian R, Moein S, et al. The effect of hormonal and non-hormonal contraceptive methods on the antioxidant content of human milk. Acta Medica Iranica 2004;42(6):427-431.
16. Subakir SB, Abdul MO, Nakano M. Oxidative stress, vitamin E and progestin Break through bleeding. Hum Reprod 2000;S3:18-23.
17. Ciavatti M, Renaud S. Oxidative status and oral contraception: Its relevance to platelet abnormalities and cardiovascular risk. Free Rad Biol & Med 1991;10:325-338.
18. Wagner KH, Kamal-Eldin A, ElmadfaI, Gamma-tocopherol-an underestimated vitamin? Am Nutr Metab 2004;48:169-188.

Authors:

1. Akinloye O,
   Department of Chemical Pathology,
   College of Health Sciences,
   Ladoke Akintola University of Technology, Osogbo, Nigeria
2. Oyabiyi SA,
   Department of Medical Laboratory Sciences,
   Faculty of Basic Medical Sciences,
   Ambrose Alli University, Ekpoma, Nigeria
3. Oguntibeju OO,
   Department of Biomedical Sciences,
   Faculty of Health & Wellness Sciences,
   Cape Peninsula University of Technology,
   Bellville, South Africa
4. Arowojolu AO,
   Department of Obstetrics & Gynaecology,
   College of Medicine,
   University of Ibadan, Ibadan, Nigeria.