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## Original Research Article

## Evaluation of the activities of some antioxidant enzymes and lipid profile in the women using contraceptives

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## ABSTRACT

**Background:** Contraception provides a means through which conception can be regulated by interfering with the normal process of ovulation, fertilization and implantation. Few public health interventions are as effective as family planning programs at reducing the mortality and morbidity of mothers and infants and have such a breadth of positive impacts.

**Aim:** This research focus on evaluation of the lipid profiles and activities of antioxidant enzymes in women using contraceptives, which are the main line of defense against free radicals.

**Materials and Methods:** One hundred and Eighty (180) women were recruited from Ekiti State University Teaching Hospital, Ado-Ekiti and divided into 6 groups based on the method of contraceptives used. Group 1 served as control (women using natural planning), Group 2 (women using oral pills), Group 3 (women using intra uterine device), Group 4 (women using implant), Group 5 (women using injection) and Group 6 (women using condom). Parameters including Body mass index (BMI) and the lipid profiles (Total Cholesterol (TC), High density lipoprotein (HDL), Low density lipoprotein (LDL) and Triglycerides (TG) were estimated in the plasma of all the test groups. The results showed significant increase in the BMI of women using pills, IUD, Implant and Injection ( $p < 0.05$ ). Significant decrease were observed in the Total cholesterol of Injection, IUD and Tablet users ( $p < 0.05$ ), significant decrease were observed in HDL of women using Tablet and Implant.

**Results:** There was no significant decrease in the plasma level of women with the age range of 38-48 and activity of antioxidant enzymes, a significant increase in the Body mass index of these women ( $p < 0.05$ ) and slight increase in the weight of women using hormonal contraceptive when compared with the natural family planning method which served as the control.

**Conclusion:** It can be said that lipid profile and antioxidant enzymes were implicated with the use of contraception, depending on the method used.

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## 1. Introduction

The rate of population growth in any country of the world is essentially the difference between the birth rate and the death rate, since international migration is today negligible. The current birth rate in western European countries is 14

to 20 per 1,000 populations, with an average of two to three children born to each woman by the end of child bearing. The death rate is 7 to 11 per 1,000, and the expectation of life at birth is about 70 years. The large development of sanitation and scientific and technological advances in many areas, such as public health, medicine, agriculture, and industry has led to a decline in the death rate and an increase in fertility rate. These gave a strain on global and

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environmental resources which hindered the rate of growth of the countries due to overpopulation. Some changes in social and cultural patterns also explain the decline in the population birth rates in terms of why people were willing to modify their sexual behavior in order to have fewer children. Among the factors underlying this particular change in attitude was a change in the economic consequences of child-bearing.

Since the burden of childcare falls primarily on women, their rise in status is probably an important element in the development of an attitude favoring the deliberate limitation of family size. Hence this problem was attacked by developing new methods of fertility regulation, birth control (contraception) and implementing programs of voluntary family planning widely and rapidly throughout the world. Contraception method is used worldwide for over birth control or unwanted pregnancies. Many workers have suggested that the use of contraceptives is beneficial but also have some side effects too. The researchers believe that the widespread use of hormonal contraceptive provides an opportunity for assessing the influence of estrogens and progesterone on various biochemical parameters among users.

There are different kinds of birth control that act at different points in the process. Birth control has been used since ancient times, but effective and safe methods of birth control only became available in the 20th century. Planning, making available and using birth control is called family planning. Some cultures limit or discourage access to birth control because they consider it to be morally, religiously, or politically undesirable.<sup>1</sup>

Barrier methods, hormonal methods and sterilization, plus new contraceptive products or methods are available today. These are new implants, a medicated intrauterine device, contraceptive vaginal rings, transdermal patches and several new regimes of combined oral contraceptives. These new or improved methods have been developed to expand the contraceptive choices available to women and men, as well to respond to the unmet need for contraceptives and to this protection against sexually transmitted disease.<sup>2</sup>

The use of contraceptives to prevent unwanted pregnancies, as well the use of barrier methods to prevent sexual transmitted diseases (STDs) has got a significant impact in modern lives. Both in the developing as well as developed countries, contraception has played a big role in women empowerment and a choice to better plan and control their lives, sexuality and their families planning as a whole.

Factors such as financial implications, social implications, and sexual empowerment or to keep a job may influence the decision of when to have a baby and hence compel the use of measures to prevent undesired pregnancy. Recognizing its impact on the present society, the US Centers for Disease Control and Prevention (CDC)

announced it as among the 10 greatest milestones of the twentieth century. The use of contraceptive is an effective means to prevent unwanted pregnancy and maternal mortality. If used properly, it can avoid more than 90% of abortion related and 20% of obstetric related death in the world.<sup>3</sup>

ROS could also be said as chemically reactive molecules derived from molecular oxygen and formed as a by-product of aerobic metabolism. During energy conversion, ROS are produced as a by-product of oxidative phosphorylation which is presumed to be the major source of superoxide production. This intracellular reduction-oxidation imbalance called oxidative stress, can subsequently contribute to the development and/or progression of cardiovascular diseases such as atherosclerosis, ischemia-reperfusion injury, chronic ischemic heart disease, cardiomyopathy, congestive heart failure, and even ensuing arrhythmias.<sup>4</sup>

Antioxidants are enzymes or compounds that scavenge or reduce the presence of free radicals. These compounds are exogenous or endogenous in nature which either prevents the generation of toxic antioxidants, intercept any that are generated and inactivate them and thereby block the chain propagation reaction produced by these oxidants. Antioxidants may favorably influence plaque stability. These compounds may be synthesized in the body or obtained from diet. The amount of protection by any one antioxidants will also depend on its concentration, its reactivity towards the particular reactive oxygen species being considered, and the status of the antioxidants defense by chelating transition metals and preventing them from catalyzing the production of free radicals in the cell.

Many enzymes, transporters, structural proteins, antigens, adhesions, and toxins are lipoproteins. Apolipoproteins are embedded in the membrane, both stabilizing the complex and giving it functional identity determining its fate. The interaction of the proteins forming the surface of the particles (with enzymes in the blood; with each other; and with specific proteins on the surfaces of cells) determines whether triglycerides and cholesterol will be added to or removed from the lipoprotein transport particles.<sup>5</sup>

The effects of estrogens and progesterone on lipoprotein metabolism are of importance because of the involvement of lipoproteins in endothelial damage and arterial occlusions. Low-density lipoprotein-cholesterol (LDL-C) is mainly implicated in the endothelial damage because of its ability to form atherosclerotic plaque. High-density lipoprotein – cholesterol (HDL-C) has pleiotropic effects that prevent or alleviate endothelial damage. The alterations in lipid metabolism that occur with the use of hormonal contraceptives have aroused considerable concern that hormonal contraceptives might increase the risk of premature atherosclerosis. Low dose hormonal contraceptive did not pose the damage of increasing the

incidence of cardiovascular and thromboembolic diseases.<sup>6</sup> Hence, the present study was conducted to evaluate the activities of some antioxidant enzymes and lipid profile in women using contraceptives.

## 2. Materials and Methods

The present study was conducted in Ekiti State University Teaching Hospital, Ado-Ekiti, Ekiti state, Nigeria. Subjects used for this study are made of 180 women using different contraceptive methods. The subjects are made up of 6 different groups, 30 women in each group of age range 38–48 years.

Group 1 is made up of 30 samples which is the control i.e., women using natural family planning, Group 2 is made up of 30 samples of women using oral pills, Group 3 is made up of 30 samples of women using intra uterine device, Group 4 is made up of 30 samples of women using implant, Group 5 is made up of 30 women using injection, Group 6 is made up of 30 women using condom.

An interview was conducted to obtain the socio demographic and reproductive health of the subjects. These were obtained by the use of questionnaire, which provides all the relevant information that ensured the choice of the right subjects and exclusion of the subjects that were not fit for the study. Height (in meters) was measured without shoes with a wall-mounted ruler. Weight (in kilogram) in light clothing was measured with a bathroom (Zhongshan Camry Electronic Co. Ltd, Guandong, China). The body mass index was calculated using the formula;  $weights(Kg) \div heights(m^2)$ .

A letter of identification from the Department of Biochemistry, Ekiti State University, Ado-Ekiti was used in pre-survey visits to hospitals to obtain permission from the hospitals authorities. An ethical clearance was also obtained from Ekiti State University Teaching Hospital, Ado-Ekiti.

### 2.1. Sample collection

All subjects in each group while resting had 10ml of their venous fasting blood samples drawn by venipuncture from each of the women into a lithium heparinized anticoagulant bottles. The blood was collected from the site of puncture and was immediately centrifuged at 4000 revolutions per minute (ppm) for 15 minutes, and separated to obtain plasma. The samples were then analyzed to obtain the concentrations of all the desired parameters in this study. The activity of SOD, GPx, CAT were estimated, also the concentration of TC, HDL, LDL, TG were assayed

The activity of SOD was estimated by the method of Kakkar et al.<sup>7</sup> Glutathione (GPx) was assayed by the method of Rotruck et al.<sup>8</sup> The catalase activity (CAT) was assayed by the method of Sinha.<sup>9</sup> Plasma total cholesterol concentrations, Plasma high density lipoprotein cholesterol, Plasma triacylglyceride concentrations were

estimated spectrophotometrically according to the method of Burtis and Edward.<sup>10</sup> Plasma Low-density lipoprotein-cholesterol was analyzed mathematically using Friedwald.

### 2.2. Statistical analysis

The data was analyzed using one analysis of variance (ANOVA) to compare the data obtained from the experiment to those of the control subjects.

## 3. Results

Table 1 shows the Anthropometric measurement of women using different contraceptive, and a significant change was observed in their body mass index compared to those using natural family planning methods (which is the control). A significant increase was observed in the value of Tablet, IUD, Implant and Injection with the value  $27.11 \pm 0.3$ ,  $31.01 \pm 0.29$ ,  $30.5 \pm 0.30$  and  $28.90 \pm 0.32$  respectively and a significant decrease was observed in the value of condom with the value  $24.87 \pm 0.28$  compared to the control with the value  $24.61 \pm 0.30$ .

Table 2 show the effect of different contraceptives on the Antioxidant enzymes [Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), and Catalase] in women. Non-significant changes were observed in the level and activity of Antioxidant enzymes when compared to the control. In SOD, a slight decrease was observed in the values of injection ( $1.019 \pm 0.363$ ) and tablet ( $0.884 \pm 0.249$ ) and significant increase was observed in the value on IUD ( $1.194 \pm 0.292$ ) when compared with the control with the value ( $1.178 \pm 0.352$ ). For GPx, a slight decrease was observed in the value of IUD ( $0.0314 \pm 0.0034$ ) and tablet ( $0.0304 \pm 0.0012$ ) also, a non-significant increase was observed in the value of injection ( $0.0344 \pm 0.0039$ ) and implant ( $0.034 \pm 0.024$ ) with the value of the Control as ( $0.0336 \pm 0.0044$ ). For Catalase, there was a slight decrease in the value of IUD ( $0.0033 \pm 0.0149$ ), and a slight increase in the value of injection ( $0.0033 \pm 0.0149$ ), tablet ( $0.0136 \pm 0.0149$ ) and implant ( $0.023$ ) when compared with the value of the Control ( $0.0072 \pm 0.0032$ ).

Table 3 show the results of the triglycerides, total cholesterol, low density lipoprotein and high density lipoprotein of women using different contraceptives. Significant change was observed in TC, HDL and LDL compared to those using natural family planning method (which is the control). For TC, there was a significant decrease ( $p = 0.011$ ) in the value of the injection, IUD and tablet with the value  $3.750 \pm 0.25$ ,  $4.240 \pm 0.27$ ,  $3.41 \pm 0.4$  and  $4.075 \pm 0.44$  respectively and there was a significant increase in the value of implant with the value 5.4 compared to the control with the value  $4.825 \pm 0.67$ . For HDL, there was a significant decrease ( $p = 0.030$ ) in the value of tablet and implant with the value  $1.125 \pm 0.17$  and 1.3 respectively and there was a significant increase in the value of injection

and IUD with the value  $1.5 \pm 0.12$ ,  $2.01 \pm 0.3$  and  $1.640 \pm 0.13$  compared to the value of control with the value  $1.375 \pm 0.34$ . For LDL, there was a significant decrease ( $p = 0.013$ ) in the value of injection, IUD, tablet and implant with the value  $1.8 \pm 0.35$ ,  $2.020 \pm 0.13$ ,  $2.375 \pm 0.73$ ,  $1.01 \pm 2.34$  and  $1.4$  respectively compared to the control with the value  $3.000 \pm 0.50$ .

#### 4. Discussion

BMI differences were statistically significantly different between control and other contraceptives users. The control group and the condom groups had normal BMI. There is significant increase in the value of Tablet, IUD, Implant and Injection with the value  $27.11 \pm 0.3$ ,  $31.01 \pm 0.29$ ,  $30.5 \pm 0.30$  and  $28.90 \pm 0.32$  respectively. According to WHO, BMI between 25.0–29.9 are classified as pre-obesity while BMI between 30.0–34.9 are classified as obesity class 1.<sup>3</sup> According to this study, tablet and injection users are at the pre-obese stage, while implant and injection users are at the obesity class 1 stage.

Piccoli observed that Oral contraceptive use was a predictor for weight increase among Swedish women in a long-term study.<sup>4</sup> In two separate surveys, one in the UK and the other in the US, 73% and 50% respectively, believed that Oral contraceptives could lead to weight gain.<sup>11</sup>

Hormonal Contraceptive use indirectly affects cardiovascular risk through mechanisms involving weight gain and obesity. Obesity is said to reduce the efficacy of contraceptives because of their pharmacokinetic alterations. However, obesity is a well-established cardiovascular risk factor, associated with cardio metabolic risk factors<sup>4</sup> including hypertension, type 2 diabetes, and high serum cholesterol.<sup>12</sup> Estrogen affect the cardiovascular system through its impact on cardiovascular risk factors such as lipid profile.<sup>12</sup> The relationship between abnormal lipid levels and risk for coronary heart disease in all regions of the world has been established.<sup>13</sup>

In Table 1 a significant increase was observed in the body mass index of women using contraceptive methods other than the normal family planning method. The observations in table 1 showed that there was a significant increase in the weight of women using contraceptives especially the hormonal contraceptive due to high levels of estrogen which increases appetite and promote fluid or water retention in women using progestin-only contraceptive. This in agreement with Nicole.<sup>14</sup>

Women using condom as a contraceptive method showed a BMI of  $(24.89 \pm 0.28)$  when compared to the natural family planning method  $(24.61 \pm 0.30)$  from Table 3. This showed that there is no significant change in weight since condom does not contain any hormone hence there's no direct way it would affect weight.

Observations deduced from Table 1 also indicates that the use of implants as a contraceptive method over time

increases body weight. Although various research shows that the use of implants does not lead to the accumulation of body fat while some argue that the use of implants increases body weight, This in agreement with pervious work done by Laureen et al.<sup>15</sup> who state that implants release a synthetic form of the hormone progesterone called progestin which also causes water retention making women feel bloated and slightly heavier.

Changes in the level and activity of some antioxidant enzymes (Superoxide Dismutase, Glutathione peroxidase, and Catalase) over time in women using contraceptives.

In Table 2, there was non- significant change observed in the level and activity of antioxidant enzymes in women when compared to the control (women using natural family planning method) for SOD and for GPx. Although there was a slight decrease in the level and activity of SOD and GPx in women using hormonal contraceptives (both tablet for SOD and for GPx while injection for SOD and when compared to the control for SOD and for GPx. This result is in agreement with previous work done by Palan et al.<sup>16</sup> who reported that hormonal contraceptives depletes total antioxidant activity. According to the antioxidant effects of oestrogens are antagonized by progestins via the activation of NADPH oxidase and inhibition of the expression and activity of manganese superoxide dismutase (MnSOD) and extracellular SOD. Therefore, it implies that the counteractive effect of progestin would result into decrease in the serum total antioxidant status especially in women taking either combined oral or combined injectable contraceptives. This study also correlates with Fallah et al.<sup>17</sup> Who stated that, oral contraceptives may stimulate or reduce the activities of GPx and SOD enzymes respectively. This may be due to an effect of these pills on bone marrow erythroblast maturation via stimulation or inhibition of the synthesis of new active GPx and SOD molecules or may be a result of increased frequency of an allele of the GPx and SOD enzymes. It is suggested that these alterations in GPx and SOD, activities may be related to their probable protective effects in response to various pathological and physiological properties of oral contraceptives.

From Table 2, it was observed that the activity of catalase was not significantly affected with P value (0.102) from the result shown in Table 2 showing that the type of contraceptive used has little or no significant effect on the activity of catalase as an antioxidant.

Table 3 show the lipid profile of all the test groups. Significant change was observed in TC, HDL and LDL compared to those using natural family planning method (which is the control). For TC, there is a significant decrease in the value of the injection, IUD and tablet with the value respectively and there was a significant increase in the value of implant with the value 5.4 compared to the control with the value. For HDL, there was a significant decrease ( $p = 0.030$ ) in the values of tablet and implant method and there

**Table 1:** Anthropometric measurement of females using contraceptive

Group	BMI	Age range	Duration of usage
Natural (Control)	24.61±0.30 <sup>a</sup>	38-48 yrs	4.02±0.01
Oral	27.11±0.31 <sup>b</sup>	38-48 yrs	4.71±0.02
IUD	31.01±0.29 <sup>b</sup>	38-48 yrs	5.99±0.01
Implant	30.5±0.30 <sup>c</sup>	38-48 yrs	4.10±0.01
Injection	28.90±0.32 <sup>c</sup>	38-48 yrs	3.19±0.02
Condom	24.87±0.28 <sup>a</sup>	38-48 yrs	6.89±0.02

BMI- Body Mass Index, IUD- Intrauterine Devices, Superscript a,b and c - indicates significant differences between the values.

**Table 2:** Antioxidant enzyme levels in women using different contraceptive

Test PAR	Control	Injection	IUD	Tablet	Implant
SOD	1.178±0.352a	1.019± 0.363a	1.194± 0.292a	0.884±0.249a	
GPX	0.0336± 0.00044b	0.0344±0.0039b	0.0314±0.00034b	0.0304± 0.0012b	0.034±0.024b
CAT	0.0072±0.0035b	0.0033±0.0149b	0.0033±0.0149b	0.0136±0.0149	0.023

P > 0.05, superscript a and b indicate a slight difference in values.

Results are expressed as mean and standard deviation

**Table 3:** Showing the results of the triglycerides, total cholesterol, low density lipoprotein and high density lipoprotein of women using different contraceptives

Test Parameters	TC	TG	HDL	LDL
Natural (Control)	4.240 ± 0.27Y	0.950 ± 0.3X	1.375 ± 0.34X	3.000 ± 0.50X
IUD	4.240 ± 0.27Y	1.360 ± 0.53X	1.640 ± 0.13Y	2.020 ± 0.13Y
Tablet	4.075 ± 0.44Y	1.3 ± 0.39X	1.125 ± 0.17Y	2.375 ± 0.73Y
Implant	5.33 ± 0.096Y	1.73 ± 0.095X	1.38 ± 0.08Y	1.13 ± 0.222Y
Injection	3.750 ± 0.25Y	1.6 ± 0.4X	1.5 ± 0.12X	1.8 ± 0.35Y
Condom	3.41±0.4	0.70±0.2	1.01±0.3	3.01±2.34

Values are expressed as mean and standard deviation. p< 0.05, Superscript X and Y

was a significant increase in the value of injection and IUD with the values compared to the value of control with the values. For LDL, there was a significant decrease ( $p = 0.013$ ) in the value of injection, IUD, tablet and implant compared to the control.

It has been observed that the significant increase observed in low density lipoprotein and high density lipoprotein exposes women using hormonal contraceptives to the risk of having stroke and myocardial infarction.<sup>18</sup>

Elevated serum level of lipid is probably the most important biochemical risk factors of arteriosclerosis. In the liver triglycerides synthesis is enhanced by oestrogen and inhibited by androgen and these triglycerides are partly brought into circulation as low density lipoproteins. When a contraceptive method containing an oestrogen and progesterone is introduced, the resultant effect is not predictable. The clinical significance of the observation is supported by the fact that arteriosclerosis begins early in life and is accelerated in the presence of high cholesterol and serum triglycerides.<sup>19</sup>

The low TC observed in this study agreed with the finding made by.<sup>20</sup> The significant elevation observed in the HDL of the women using injectable hormonal contraceptive and the significant reduction in the LDL level of these women was also observed in a finding made by Crome et

al.<sup>21</sup> he observed decreased TC, LDL and increased HDL in women taking medoxy progesterone.

Asare, reported in his research on Ghanaian community that there is a significant increase in TC among women using hormonal contraceptives with P-value 0.002, significant increase TG among women using hormonal contraceptives with P- value 0.026 and significant decrease in LDL among women using hormonal contraceptives with P- value 0.018 compared to the control.

## 5. Conclusion

The findings of this study revealed a slight decrease in the total antioxidant level among hormonal contraceptives users. This suggests that women taking hormonal contraceptives are at risk of oxidative stress related disease e.g arteriosclerosis with use over time. Though alteration in various metabolic processes and antioxidant status associated with the use of hormonal contraceptive remains controversial.

Also, a significant change was observed in the lipid profile of women such as total cholesterol, high density lipoprotein and low density lipoprotein in Injection, IUD, Tablet and Implant contraceptive users. It further suggests some potential cardiovascular disease risk with Hormonal

Contraceptive use through an indirect mechanism of BMI increase, which appears to be a more consistent marker in the literature in relation to Hormonal Contraceptive use than the lipid profile. However further research is suggested.

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None.

## 7. Conflict of Interest

None.

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