



**STUDIES ON SOME PLASMA BIOCHEMICAL PARAMETERS IN SHORT AND LONG TERM USERS OF ORAL CONTRACEPTIVES CONTAINING LOWER DOSES OF ESTROGEN AND PROGESTIN COMPOSITION IN LAGOS, NIGERIA**

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**ABSTRACT**

The aim of this study was to assess the concentrations of some plasma biochemical parameters in short and long term users of oral contraceptives containing lower doses of estrogen and progestin composition as compared with that of non contraceptive users (control). 5ml blood specimen each was collected into lithium heparinized anticoagulated bottle from one hundred and fifty apparently healthy volunteers who were grouped into three with group one consisting of fifty volunteers who have been on these oral contraceptives for a period of  $\leq 5$  months (short term users) aged 31-35 years (experimental group one), group two consisting of another fifty volunteers who have been on these oral contraceptives for a period of  $\geq 2$  years (long term users) aged 31-35 years (experimental group two) and group three consisting of the remaining fifty volunteers who were not on oral contraceptives or any other medications before and during the course of this study aged 31-35 years (control group). The plasma obtained from the spun blood specimens were used for the quantitative measurement of creatinine, urea, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin and C-reactive protein. The results showed that the mean values of plasma alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin and C-reactive protein were significantly elevated ( $p \leq 0.05$ ) in the long term users of these oral contraceptives (experimental group two) as compared with that of non contraceptive users (control group) while the mean values of plasma creatinine and urea were not significantly different ( $p \geq 0.05$ ) as compared with that of non contraceptive users (control group). However, the results went further to show that there were no statistical significant differences ( $p \geq 0.05$ ) in the mean values of all the plasma biochemical parameters measured in the short term users of these oral contraceptives (experimental group one) as compared with that of non contraceptive users (control group). In conclusion, this study has affirmed that plasma alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin and C-reactive protein concentrations are elevated in long term users of oral contraceptives containing lower doses of estrogen and progestin composition for a period of  $\geq 2$  years aged 31-35 years. It is therefore recommended that long term users of oral contraceptives containing lower doses of estrogen and progestin composition should embark on routine laboratory investigations on these plasma biochemical parameters with the aim to bring the concentrations to normal value if found elevated.

**KEYWORDS:** Studies, plasma biochemical parameters, short term users of oral contraceptives, long term users of oral contraceptives, Lagos, Nigeria.

**INTRODUCTION**

At some time in the life of a couple, it is expedient to prevent sexual intercourse from resulting into pregnancy, this may however, be due to the fact that such couple may not be willing to have a child at that particular point in time for one or more reasons. At such times, sexual intercourse which ordinarily would have resulted into pregnancy is prevented via the use of oral contraceptive which is defined as a medication taken by mouth for the

purpose of birth control. The use of oral contraceptive which is often referred to as "family planning" has significantly improved infant, maternal and family health.<sup>[1,2]</sup>

Contraceptive agents were designed scientifically years back when into various animals, corpus luteum extracts were injected and discovered to prevent ovulation.<sup>[3]</sup> In 1934, the active component of corpus luteum was

isolated and structurally revealed as Pregn-4-ene-3, 20-dione (1) progesterone, but since the oral activity of progesterone is very low its daily intramuscular injection for continuous contraceptive purposes i.e. the inhibition of ovulation in human female is therefore impracticable a situation which thus influenced the modification of its basic molecular structure with the coming up of oral contraceptives<sup>[4]</sup> which was originally launched as a pill to be used containing a combination of nor-19-progestin with some quantity of synthetic estrogen.<sup>[5]</sup> This quest was born by Margaret Sanger in the middle of the 20<sup>th</sup> century.<sup>[6]</sup>

In America oral contraceptive pills remain the most liked method of contraception with 16% of women between the ages of 15-44 years using it.<sup>[7]</sup> However, in most developing countries, including Nigeria, the use of contraceptive pills is solicited for by government as well as different organizations<sup>[8]</sup> this probably may have triggered its increase use in India over the decade as reported by.<sup>[9]</sup> It has also been reported that millions of women within the reproductive age all over the world make use of oral contraceptives<sup>[10]</sup> which are reported to have safe therapeutic doses, but caution is required for their prolong usage with particular reference to their concentration which may be genotoxic in humans.<sup>[11]</sup> It was reported by some researchers long ago that the first contraceptive pill (Enovid) which was far from perfection has high doses of hormones such as 10,000microgram of progestin and 150 microgram of estrogen which according to them was responsible for its potency in the prevention of pregnancy. However, these researchers failed to realize earlier that contraceptive pills that contain lower doses of hormones such as 50-150microgram progestin and 20-50 microgram of estrogen were also as potent in preventing pregnancy as compared to those with higher doses.<sup>[12,13]</sup>

Despite the campaign by government in most developing countries which has led to increase in the use of oral contraceptives in such countries, particularly Nigeria, documented reports on the comparison of the status of these plasma biochemical parameters in short term users for a period of  $\leq 5$  months and long term users for a period of  $\geq 2$  years within the age range of 31-35 years respectively in the studied community are not available hence the aim of the present study.

#### MATERIALS AND METHODS

A total of one hundred and fifty apparently healthy volunteers were recruited for this study and grouped into three: Group one consists of fifty volunteers who have being on oral contraceptives containing lower doses of estrogen and progestin composition for a period of  $\leq 5$  months referred to as short term users (experimental group one) aged 31-35 years. Group two consists of another fifty volunteers who have being on oral contraceptives containing lower doses of estrogen and progestin composition for a period of  $\geq 2$  years referred to as long term users (experimental group two) aged 31-

35 years. Group three consists of the last fifty volunteers with no evidence of oral contraceptives usage and/or any other medications before and during the course of this study aged 31-35 years (control group). Reports on the medical history and social activities of the recruited volunteers (experimental groups one and two) who were all attending family planning clinics in Lagos state of Nigeria were collected and they were all confirmed to be free from hypertension, obesity, cigarette smoking and alcohol abuse before and during the course of this study thus ruling out the possibility of any effects of their lifestyle variables on the obtained results.

After seeking the consents and approval from these volunteers i.e. control and experimental groups, 5ml blood specimen was collected from each of them into lithium heparinized anticoagulated bottles respectively. Thereafter each of the blood specimens was spun for 10 minutes at 1,500 revolution/minute using Gulfex Medical and Scientific Macro Centrifuge model 800D England and the plasma obtained used for the quantitative measurement of the following biochemical parameters using S23A13192 model spectrophotometer: creatinine, Jaffe reaction method as described by Randox Laboratories Limited, 55, Diamond Road, Crumlin, County Antrim, BT294QY, United Kingdom<sup>[14,15]</sup>, urea, urease- berthelot method, as described by Randox Laboratories Limited, 55 Diamond Road, Crumlin, County Antrim, BT294QY, United Kingdom<sup>[16-19]</sup>, alanine aminotransferase (ALT), colorimetric method as described by Randox Laboratories Limited, 55, Diamond Road, Crumlin, County Antrim, BT294QY, United Kingdom<sup>[20,21]</sup>, aspartate aminotransferase (AST), colorimetric method as described by Randox Laboratories Limited, 55, Diamond Road, Crumlin, County Antrim, BT294QY, United Kingdom<sup>[22,23]</sup>, alkaline phosphatase (ALP), colorimetric endpoint method as described by Teco Diagnostics, 1268 N. Lakeview Ave. Anaheim, CA 92807, 1-800-222-9880<sup>[24]</sup>, total bilirubin, Jendrassik and Grof method as described by Randox Laboratories Limited, 55, Diamond Road, Crumlin, County Antrim, BT294QY, United Kingdom<sup>[25]</sup> and C-reactive protein (CRP), latex turbidimetry method as described in the manual of Spin-react Diagnostic kit, Spain.<sup>[26-29]</sup>

**STATISTICAL ANALYSIS:** The results of this study were expressed in mean and standard deviation, while the differences between the subjects (control and experimental groups) were assessed using the Student's "t" test. The results were considered statistically significant at  $p \leq 0.05$ .

#### RESULTS AND DISCUSSION

In this study the mean values of the biochemical parameters in short term users of oral contraceptive containing lower doses of estrogens and progestin composition i.e. for a period of  $\leq 5$  months (experimental group one) and long term users of oral contraceptive containing lower doses of estrogens and progestin

composition i.e. for a period of  $\geq 2$  years (experimental group two) within the age range of 31-35 years respectively were compared with that of the non users of oral contraceptives (control group) as shown in Tables 1 and 2 while the number and percentages of short and long term users of the oral contraceptives containing lower doses of estrogens and progestin composition with abnormal plasma biochemical values are as shown in Table 3.

The results from this study showed that the mean values of plasma creatinine, plasma urea, plasma alanine aminotransferase (ALT), plasma aspartate aminotransferase (AST), plasma alkaline phosphatase (ALP), plasma total bilirubin and plasma C-reactive proteins (CRP) in short term users of the oral contraceptive i.e.  $\leq 5$  months aged 31-35 years (experimental group one) are not significantly different statistically ( $p \geq 0.05$ ) as compared to the mean values of non users of oral contraceptives (control group) aged 31-35 years as shown in Table 1.

However 6%, 2%, 8%, 10% and 0% of the short term users of the oral contraceptives containing lower doses of estrogens and progestin composition had elevated plasma alanine aminotransferase (ALT), plasma aspartate aminotransferase (AST), plasma alkaline phosphatase (ALP), plasma total bilirubin and plasma C-reactive protein (CRP) concentrations than the existing reference range maximum of 12U/I, 12U/I, 35IU/L, 17 $\mu$ mol/l and 6mg/L respectively as shown in Table 3.

These findings which are however, not statistically significant have affirmed that short term ( $\leq 5$  months) usage of oral contraceptives containing lower doses of estrogens and progestin composition has no effect on the renal and liver functions.

Alanine aminotransferase (ALT) is an enzyme that is found mainly in the liver and when released into the bloodstream may suggest the presence of damage to the liver as a result of medication, infection and/or injuries.

The mean value of plasma alanine aminotransferase (ALT) in the long term users of the oral contraceptives containing lower doses of estrogens and progestin composition i.e. for a period of  $\geq 2$  years aged 31-35 years (experimental group two) was significantly higher statistically ( $p \leq 0.05$ ) as compared to the mean value of non users of oral contraceptive (control group) aged 31-35 years as shown in Table 2 with 60% of them found to have plasma alanine aminotransferase (ALT) concentration greater than the existing reference range maximum of 12U/I as shown in Table 3. The finding in this study with respect to the significant increase in the mean value of plasma concentration of alanine aminotransferase (ALT) may be ascribed to the prolong use ( $\geq 2$  years) of the oral contraceptive pills which is most likely to pose challenges on the normal physiological function and integrity of the liver by

inducing liver injury which is complemented by the significant release of alanine aminotransferase (ALT) due to hepatocellular leakage. Prolong users of oral contraceptives containing lower doses of estrogens and progestin composition may therefore be at risk of liver disease as confirmed in this study.

Aspartate aminotransferase is an enzyme that is also mainly produced in the liver as well as in red blood cells, skeletal and cardiac muscles and when released into the bloodstream may be suggestive of acute liver damage, obstruction, hepatitis or cirrhosis.

The mean value of plasma aspartate aminotransferase (AST) in the long term users of the oral contraceptive containing lower doses of estrogens and progestin composition i.e. for a period of  $\geq 2$  years aged 31-35 years (experimental group two) was significantly higher statistically ( $p \leq 0.05$ ) as compared to that of the non users of oral contraceptives (control group) as shown in Table 2 with 50% of them found to have plasma aspartate aminotransferase (AST) concentration greater than the existing reference range maximum of 12U/I as shown in Table 3. The finding in this study with respect to the significant increase in the mean value of plasma concentration of aspartate aminotransferase (AST) may be ascribed to the prolong use ( $\geq 2$  years) of the oral contraceptive pills which is most likely to pose challenges on the normal physiological function and integrity of the liver by inducing liver injury which is complemented by the significant release of aspartate aminotransferase (AST) due to hepatocellular leakage. Prolong users of oral contraceptives containing lower doses of estrogens and progestin composition may therefore be at risk of liver disease as confirmed in this study.

The mean value of plasma alkaline phosphatase (ALP) in long term users of the oral contraceptive containing lower doses of estrogens and progestin composition i.e. for a period of  $\geq 2$  years aged 31-35 years was significantly higher statistically ( $p \leq 0.05$ ) as compared to that of the non users of oral contraceptives (control group) as shown in Table 2 with 52% of them found to have plasma alkaline phosphatase (ALP) concentration greater than the existing reference range maximum of 35IU/L as shown in Table 3. The finding in this study with respect to the significant increase in the mean value of plasma concentration of alkaline phosphatase (ALP) is suggestive of liver damage which may be as a result of the prolong use ( $\geq 2$  years) of the oral contraceptive pills which has thus complemented the significant release of alkaline phosphatase (ALP) indicating hepatocyte membrane loss of functional and structural integrity with subsequent hepatocellular leakage. Prolong users of oral contraceptives containing lower doses of estrogens and progestin composition may therefore be at risk of liver disease as confirmed in this study.

The mean value of plasma total bilirubin in long term users of the oral contraceptives containing lower doses of estrogens and progestin composition i.e. for a period of  $\geq 2$  years aged 31-35 years was significantly higher statistically ( $p \leq 0.05$ ) as compared to that of the non users of oral contraceptives (control group) as shown in Table 2 with 70% of them found to have plasma total bilirubin concentration greater than the existing reference range maximum of  $17 \mu\text{mol/l}$  as shown in Table 3. This finding with respect to the significant increase in the mean value of plasma concentration of total bilirubin is suggestive of hepatobiliary complications which is most likely induced by the prolong use of oral contraceptives with the subsequent inhibition of the hepatic excretory function thus leading to hyperbilirubinaemia as confirmed in this study.

The mean value of plasma C-reactive protein (CRP) in the long term users of oral contraceptive containing lower doses of estrogens and progestin composition i.e. for a period of  $\geq 2$  years aged 31-35 years was significantly higher statistically ( $p \leq 0.05$ ) as compared to that of the non users of oral contraceptives (control group) as shown in Table 2. 64% of these users were found to have plasma C-reactive protein (CRP) concentration greater than the existing reference range maximum of  $6 \text{mg/L}$  as shown in Table 3. This finding with respect to the significant increase in the mean value of plasma concentration of C-reactive protein (CRP) resulting from the prolong use ( $\geq 2$  years) of oral

contraceptive pills containing lower doses of estrogen and progestin may be suggestive of inflammation of the liver which has led to the release of interleukin-6 and other cytokines capable of triggering the increase synthesis of C-reactive protein.

The mean values of plasma creatinine and plasma urea in the long term users of oral contraceptives containing lower doses of estrogens and progestin composition i.e. for a period of  $\geq 2$  years aged 31-35 years were not significantly different statistically ( $p \geq 0.05$ ) as compared to that of the non users of oral contraceptives (control group) as shown in Table 2. These results as confirmed in this study show that long term users of oral contraceptives containing lower doses of estrogens and progestin composition i.e. for a period of  $\geq 2$  years aged 31-35 years are not exposed to any risk associated with renal dysfunction.

Regardless of the fact that various oral contraceptive formulations with marked decrease in dosage of estrogen and progestin composition have been developed in the past 30 years with the resultant low pregnancy rates in their usage as compared to those formulations having higher doses of steroid<sup>[30,31]</sup>, the findings from this study have affirmed that oral contraceptive pills irrespective of their dosage are capable of impairing liver function as well as inducing liver damage thus causing inflammation.

**Table 1: Biochemical Parameters in Short Term Users of Oral Contraceptives Containing Lower Doses of Estrogen and Progestin Composition (Experimental Group One) Compared with Non Users of Oral Contraceptives (Control Group).**

Parameters measured	Control (n=50)	Short term users (n=50)	Remark
Creatinine ( $\mu\text{mol/l}$ )	$50.00 \pm 0.72$	$50.30 \pm 0.73$	NSD
Urea (mmol/l)	$3.48 \pm 0.27$	$3.50 \pm 0.28$	NSD
ALT(U/I)	$7.00 \pm 0.43$	$7.10 \pm 0.45$	NSD
AST(U/I)	$6.70 \pm 0.38$	$6.80 \pm 0.40$	NSD
ALP (IU/L)	$20.10 \pm 0.95$	$20.04 \pm 0.94$	NSD
Total bilirubin( $\mu\text{mol/l}$ )	$5.30 \pm 0.82$	$5.38 \pm 0.84$	NSD
CRP (mg/L)	$3.20 \pm 0.22$	$3.30 \pm 0.25$	NSD

#### KEY

Values are in mean  $\pm$  SD,

NSD represents not significantly different

ALT represents alanine aminotransferase

AST represents aspartate aminotransferase

ALP represents alkaline phosphatase

CRP represents C-reactive protein

**Table-2: Biochemical Parameters in Long Term Users of Oral Contraceptives Containing Lower Doses of Estrogen and Progestin Composition (Experimental Group Two) Compared with Non Users of Oral Contraceptives (Control Group).**

Parameters measured	Control (n=50)	Long term users (n=50)	Remark
Creatinine ( $\mu\text{mol/l}$ )	50.00 $\pm$ 0.72	50.22 $\pm$ 0.73	NS
Urea (mmol/l)	3.48 $\pm$ 0.27	3.50 $\pm$ 0.29	NS
ALT(U/I)	7.00 $\pm$ 0.43	31.50 $\pm$ 1.03	S
AST(U/I)	6.70 $\pm$ 0.38	22.25 $\pm$ 0.87	S
ALP (IU/L)	20.10 $\pm$ 0.95	62.70 $\pm$ 1.72	S
Totalbilirubin( $\mu\text{mol/l}$ )	5.30 $\pm$ 0.82	34.45 $\pm$ 1.35	S
CRP (mg/L)	3.20 $\pm$ 0.22	13.22 $\pm$ 1.23	S

**KEY**

Values are in mean  $\pm$  SD

SD represents significantly different

NSD represents not significantly different

ALT represents alanine aminotransferase

AST represents aspartate aminotransferase

ALP represents alkaline phosphatase

CRP represents C-reactive protein

**Table-3: Number and Percentage of Short and Long Term Users of Oral Contraceptives Containing Lower Doses of Estrogen and Progestin Composition with Abnormal Plasma Biochemical Values.**

Parameters measured	Control (n=50)	Short term users (n=50)	Long term users (n=50)
Creatinine ( $\mu\text{mol/l}$ )	0(0)	0(0)	0(0)
Urea (mmol/l)	0(0)	0(0)	0(0)
ALT(U/I)	0(0)	3(6)	30(60)
AST(U/I)	0(0)	1(2)	25(50)
ALP (IU/L)	0(0)	4(8)	26(52)
Totalbilirubin( $\mu\text{mol/l}$ )	0(0)	5(10)	35(70)
CRP(mg/L)	0(0)	0(0)	32(64)

**KEY**

The values as presented show percentages in parenthesis

ALT represents alanine aminotransferase

AST represents aspartate aminotransferase

ALP represents alkaline phosphatase

CRP represents C-reactive protein

**CONCLUSION**

Sequel to the significant elevations of plasma alanine aminotransferase (ALT), plasma aspartate aminotransferase (AST), plasma alkaline phosphatase (ALP), plasma total bilirubin and plasma C-reactive protein (CRP) in the long term users of oral contraceptives containing lower doses of estrogen and progestin composition as compared with that of the short term users, it is expedite to conclude from this present study that prolong usage ( $\geq 2$  years) of these oral contraceptives is capable of impairing liver function with the subsequent cause of liver damage, jaundice and inflammation.

**Recommendations**

It is recommended that:

(i) Women on prolong usage ( $\geq 2$  years) of oral contraceptives containing lower doses of estrogens and progestin composition should check their liver regularly by embarking on blood liver function test.

(ii) Prolong users of oral contraceptives ( $\geq 2$  years) containing lower doses of estrogens and progestin composition should consult their doctors or health professionals for family planning alternatives if these oral contraceptives are not good for them.

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