## Original Research Article

# IMPACT OF COMBINED ORAL CONTRACEPTIVE PILLS ON CD4 COUNT, OSMOTIC FRAGILITY, TOTAL AND DIFFERENTIAL WHITE BLOOD CELLS OF USERS IN ANTENATAL CLINIC IN PORT HARCOURT



**ABSTRACT**

**Aims:** The use of hormonal contraceptives is on the increase among women of reproductive age in Nigeria. In this study, effort was made to examine the influence of combined oral contraceptive on some white blood cells parameters in women attending family planning clinic.
**Study design:**Descriptive and cross-sectional study.
**Place and Duration of Study:**The study was in University of Port Harcourt for a period of 8 weeks.
**Methodology:**Data for this study were obtained through questionnaires administered on one hundred and twenty (120) respondents who were randomly and purposively selected. Blood samples were collected and analysed using appropriate techniques. One-way ANOVA was adopted as the statistical analysis method for the study.
**Results:**It was found out from the study that combined oral contraceptive suppresses the immune system of users and may predispose them to infection.
**Conclusion:**It is concluded from the study that oral contraceptives with high oestrogen could be harmful to users while low oestrogen contraceptive has shown potentials of reversing negative effects of high oestrogen contraceptives. It is recommended from this study that women should cut down on the use of oral contraceptives especially high oestrogen content oral contraceptives.

*Keywords: [Combined, Oral,Contraceptive Pills,CD4 Count, Osmotic Fragility, White Blood Cells}*

## 1. INTRODUCTION

Since the introduction of the various contraceptive methods in the 20th century, millions of women of reproductive age group, worldwide, have made use of it to prevent unwanted pregnancies and abortions, and also to improve child birth spacing. The wide spread use of contraceptives (hormonal) provides an opportunity for assessing the influence of oestrogens and progestrogens on various biochemical parameters of the female subject (Obisesan, Adenaik, Okunla and Adenaika, 2002). It is even possible that some of the side effects of these compounds might be associated with some metabolic effects. Oral contraceptives have been implicated in many diseases such as thromboembolic disease,

myocadiac infarction, circulatory disorders, and carcinogenicity (Gaspard, 1990; Slone, Shapire and Kafmann, 1981; Cells, 1983). Furthermore, the negative effects on the liver, heart, diabetes, obesity, hypertension and high serum cholesterol levels are well documented (Gaspard, 1987).However, the biochemical profile of women on contraceptive use showed different changes in the plasma total protein, albumin, globulin and cholesterol levels (Bockner and Roman, 1986, Obisesan et al., 2002).

## 2. MATERIAL AND METHODS

Blood samples were collected from the family planning units of University of Port Harcourt Teaching Hospital (UPTH) and the volunteer female undergraduate students of University of Port Harcourt who were not on contraceptives constituted the control subjects, after approval from ethics committee of the same hospital. The ages of the subjects were in the range of 20 to 30 years and all the subjects were confirmed to be regular clients of the Family Planning Clinic of the Department of Obstetrics and Gynaecology.

i. Had no history of recent blood loss, blood disorder or pile (Hemorrhoids)

ii. No treatment for anemia in form of iron tablets or vitamin B12.

iii. No pregnancy within the last six months

iv. No cardiac or endocrine disorder.

A total of 120 women were involved in this study and grouped into three. Group I (control) consisted of 50 female volunteer subjects without contraceptives. Group II comprised 30 women with oestrogen combined oral contraceptive (methyloestranolone and methylostradiol) while group III had 40 women on norgesterol-estradiol combined oral contraceptives.

## 3. RESULTS AND DISCUSSION

### Comparison of the red blood cell osmotic fragility following the usage of oral contraceptive by women

The results of the median corpuscular fragility (MCF) for the control and test groups are shown in table 1. The results showed combined oral contraceptives did not have significant alteration to the membrane of the red blood cells.

### Evaluation of the Total White Blood Cell

As shown in figure 4, the combined oral contraceptives (group II and III) significantly increased the total white blood cell compared with the control group ($p<0.05$). Comparison between the combined oral contraceptive groups showed that group III [Norgesterol-estradiol] significantly reduced the total white blood cell with respect to group II [oestrogen] ($p<0.05$), figure 4

**Table 1: Median Corpuscular Fragility (MCF)**

| Groups | MCF |
|---|---|
| I | $0.44 \pm 0.04$ |
| II | $0.43 \pm 0.03^{NS}$ |
| III | $0.47 \pm 0.01^{NS}$ |

NS = Not significant

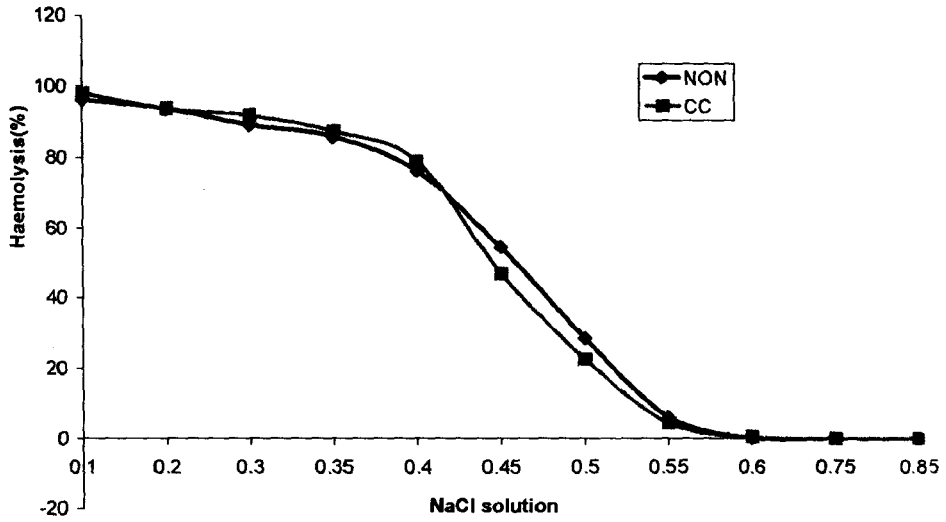



**Figure 1:** The osmotic fragility curves for the control and the combined oral contraceptive group.

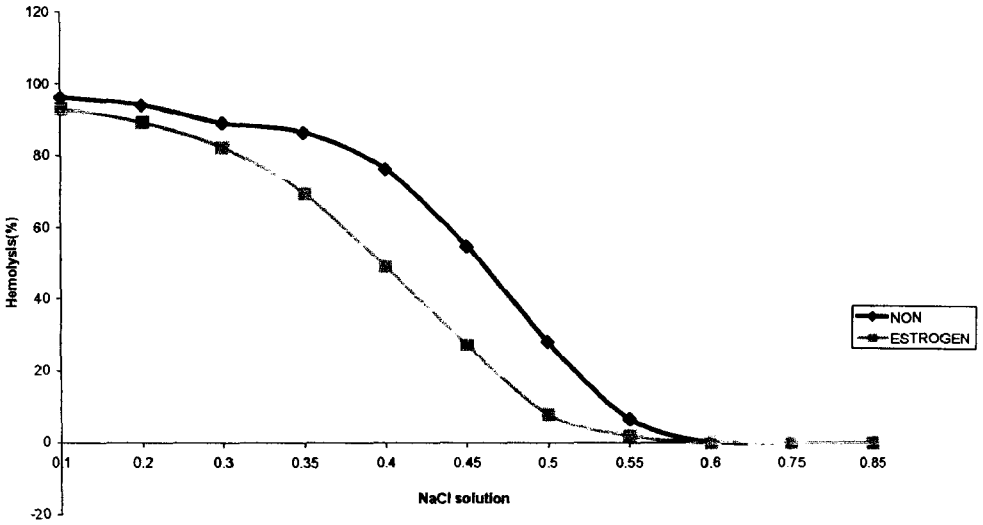



**Figure 2:** The osmotic fragility curves for the control and the combined oral contraceptive groups

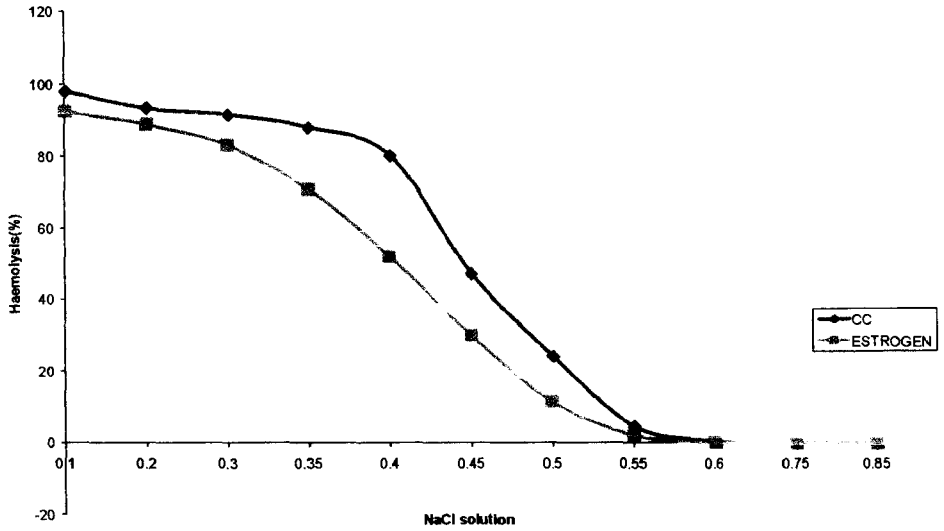



**Figure 3:** The osmotic fragility curves for the control and the combined oral contraceptive groups

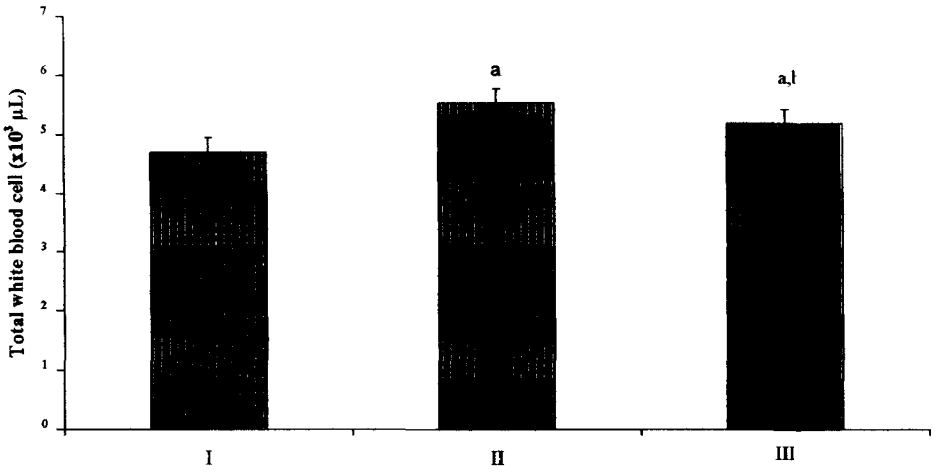



**Figure 4:** The mean total white blood cell count for the control and contraceptive groups. Values presented as mean ± SEM.a= $p<0.05$ vs group I, b= $p<0.05$ vs group II.

**Evaluation of the differential white cell count**

As illustrated in table 2, the combined oral contraceptives (groups II and III) significantly increased the percentages of naeutrophils and lymphocyte when compared with control ($p<0.05$). Similarly, the norgesterol-estradiol combined oral contraceptives (group III) significantly reduced both the neutrophil and lymphocytes when compared with oestrogen combined oral contraceptives (group II).

The combined oral contraceptives in groups II and III significantly reduced monocytes and eosinophils compared with control group ($p<0.05$). There was no significant change when comparison was done within the contraceptive groups. Similarly, combined oral contraceptives did not affect percentage of basophils significantly, table 2.

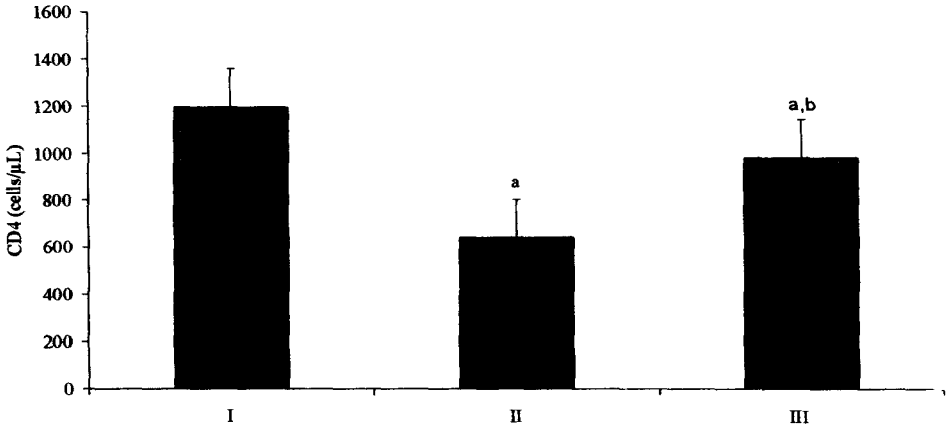
**The effect of combined oral contraceptives on $CD_4$ count**

The mean values of the $CD_4$ count (cells/µL) obtained for the control and combined oral contraceptives are presented in figure 5. Combined oral contraceptives in groups II and III significantly reduced the $CD_4$ count when compared with the control ($p<0.05$). Group III was higher than group II at $p<0.05$.

**Table 2: Comparison of the differential white blood cell count for the control and oral contraceptive groups**

| Groups | Neutrophil (%) | Lymphocyte (%) | Monocytes (%) | Eosinophil (%) | Basophil (%) |
|---|---|---|---|---|---|
| I | 39.78±0.16 | 37.21±0.34 | 11.22±0.16 | 3.96±0.18 | 0.98±0.12 |
| II | 48.70±1.87[a] | 46.20±0.22[a] | 9.50±0.30[a] | 3.35±0.12[a] | 1.11±0.11 |
| III | 46.4±1.43[a,b] | 45.34±0.7[a,b] | 9.71±0.28[a] | 3.18±0.15[a] | 0.09±0.06 |

a = significantly different from group I ($p<0.05$), b = significantly different from group II ($p<0.05$)




Groups**Figure 5:** The mean CD4 values for the control and combined oral contraceptives groups. Values presented as mean ± SEM.a= $p<0.05$ vs group I, b= $p<0.05$ vs group II.

## 3. DISCUSSION

The findings of this study showed that combined oral contraceptives have the potential to induce an increase in the production of circulating total white blood cell (TWBC), neutrophils and lymphocytes. It was also observed that the increases were prominent with oestrogen oral contraceptive. The significant increase in TWBC count, neutrophil and lymphocyte in combined oral contraceptive users however, disagrees with the findings of other investigators, who reported reductions (Ben-Hur *et al.,* 1995; Al-Chalaby, Taib and Ahmed 2006). There are conflicting reports in literature with regards to the effect of oestrogen on circulating neutrophil levels. Pincus (1965) found that subjects taking oral contraceptives with an oestrogen content of 75-150ug had higher neutrophil counts than control which is in consonance with the finding of this study, whereas Toth (1982) found that subjects taking preparations with 100ug of oestrogen had lower neutrophil counts than controls.

The significant reduction in monocyte level reported in this study is supported by the fact that sex hormones have the potentials to influence macrophage activities. As monocyte develops into macrophage, it has been reported that steroid hormones dose-dependently modulate release of tissue necrotic factor (TNF) from macrophages that influenced the function of these cells in the manifestation of their protective role of cytokine-mediated cytotoxicity (D'Agostino *et al*., 1999; Chao, Chao, Chen, Greager and Walter 2000). The significant reduction in monocyte level recorded in this study is in consonance with earlier report of Ben Hur *et al*. (1995). These workers reported a decline in monocyte counts following oestrogen therapy in menopause and suggested that oestrogen and possibly progesterone decrease monocyte numbers. Mechanism for such reduction may be due to the ability of the sex hormones to induce mitotic arrest and apoptosis in monocytes (Thongngarm, Jenkins, Ndebele and McMurray, 2003).

Macrophages play crucial roles in atherosclerosis and immunity (Masuda and Ross, 1990) and are uniquely dependent on the *milieu* to which they were exposed (Gordon and Taylor, 2005), which, can in turn be modified by oral contraceptives (Vessey, Mant, Smith and Yeates, 1986; Spitzer, Lewis, Heinemann, Thorogood and MacRae, 1996; Kirschbaum, Kudielka, Gaab, Schommer and Hellhammer, 1999; Wiegratz and Kuhl, 2004; Willis, Kuehl, Spiekerman and Sulak, 2006; Haarala *et al*., 2009; Valtonen *et al*., 2010). Importantly, monocyte-derived macrophages (MDMs) express oestrogen and androgen receptors (Murphy, Guyre, Wira and Pioli, 2009). It may be most probably inferred that the variation of internal *milieu* induced by combined oral contraceptives may affect the function of macrophages.

It was also observed that combined oral contraceptive depressed the productions of monocytes, eosinophils and the $CD_4$ level, suggestive of potential suppressing effect of immune system of users, even as the aforementioned results were prominent with oestrogen oral contraceptive. This result agrees with some other report which speculated that combined oral contraceptive pill use is associated with a trend towards lower absolute $CD_4$ count (Mami *et al*., 1996; Okumu, Makobore, Kaggwa, Kambugu and Galukande, 2013). No doubt, those significant reductions could be explained on the basis that progesterone and l7-β-estradiol can modulate activity of immune cells by activating the cells to produce cytokines (Chao, Van Alten, Greager, and Walter, 1995.; Miller and Hunt, 1998; Wilder and Elenkou, 1999).

$CD_4$ lymphocyte serves as a marker for immune status and can be used as a predictor of lowered immunity in disease conditions. The absolute number of circulating $CD_4$ lymphocyte has been shown to be a clinically useful indicator of immune function in disease conditions such as individuals infected with the human immunodeflciency virus (HIV) (Thongngarm*et al*., 2003). In infection condition such as HIV, auto immune responses may be evoked by shared structural homology between major histocompactibility (MHC) class II moecu1es and cellular humoral immune responses directed towards HIV proteins which cross react against antigens on T cells causing immune destruction. The exact mechanism of $CD_4$ reduction is said to be due to several factors; cell lysis, autoimmune mechanism, anergy, effect of super antigens, apoptosis and virus specific immune responses (Thongngarm*et al*., 2003).

There is growing evidence in the body of literature that endogenous oestrogens and progestins play a pivotal role in regulation of both hormonal and cell-mediated immunity (Grossman, 1984; Schuurs*et al*.,1994). Such biologic effects of progestins in combined oral contraceptive are speculated to be the consequence of their synergistic relationship with the estrogenic component of the oral contraceptive, confirming an outstanding reduction in $CD_4$ levels by menstrogen oral contraceptive reported in this study. Synthetic estrogens and progestins in hormonal contraceptives may therefore potentially influence immune function, probably through their suppression of endogenous hormones (Mishell, Thorneycroft, Nakamura, Nagata and Stone, 1972; Oritz, Horio, Stanczyk, Goebelsmann and Mishell, 1977) or through direct action on estrogen or progesterone receptors in immune cells.

## 4. CONCLUSION

Oral contraceptives with high oestrogen content have the tendency to suppress immune system (total white blood cell, differential white blood cell and CD4 count) of users.The supression of the immune system predisposes users to infection. We recommend the use of oral contraceptives with high oestrogen content should be discouraged because of its tendency to suppress the immune system of users. Furthermore, use of oral contraceptives with low oestrogen content should be encouraged because of its relatively low potential to cause infection. Finally, caution is necessary for the improper use of contraceptive.

## CONSENT (WHEREEVER APPLICABLE)

We obtained a written and signed consent form from the various participants before recruiting them to participate in the study.

## ETHICAL APPROVAL (WHEREEVER APPLICABLE)

Ethical clearance was obtained by from the research ethics committee of the Rivers State University before commencement of the study.
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