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Assessment of Hematological Profile, Biomarkers of Inflammation and Oxidative Stress in Women on Hormonal Contraceptives

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ABSTRACT

Hormonal contraceptives (HCs) are synthetic biochemical compounds that enable sexual activity without the risk of pregnancy. Despite their widespread use, concerns have been raised about the potential adverse effects of hormonal contraceptives (HCs) on women's health with scarce reports especially of their effects on hematological profiles, inflammation and oxidative stress biomarkers. This study was designed to investigate the effects of HCs on hematological indices, biomarkers of inflammation and oxidative stress in women. A total of 50 women from 3 health centers in Akure (Nigeria) were recruited for this study, with 25 women serving as control and 25 women on HCs (case). Blood samples were collected and analyzed using analytical kits. The results from this study indicate that women on HCs had a significant ($p < 0.001$) increase in white blood cell count, granulocytes and a significant increase ($p < 0.05$) in the platelet large cell ratio. A significant increase ($p<0.001$) in superoxide dismutase (SOD) activity and tumor necrosis factor-alpha (TNF -α) ($p<0.01$) was also observed when compared to the control group. These findings suggest that HCs may alter hematological indices and induce oxidative stress and inflammation, which can impact negatively on women's health. It is recommended that healthcare providers undertake appropriate client profiling before initiating any contraceptive choice to prevent pathological conditions, especially for those who may have pre-existing conditions and risk factors, they are however advised to opt for non-hormonal contraceptives. There is a need for further research into the potential adverse effects of HCs and the necessity for proper screening before HC administration.

Keywords: *Contraception, Hormonal contraceptives, hematological indices, red blood cell, white blood cell, platelets, oxidative stress, Inflammation*

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INTRODUCTION

Contraception is the intentional prevention of pregnancy through various artificial or natural methods. This is achieved by utilizing different contraceptive techniques and addressing unintentional infertility. Given the rising global population and environmental challenges, birth control has emerged as a crucial tool for promoting sustainability (Speidel and Jane, 2023). The primary aim of contraception is to enable individuals and couples to take control of their reproductive health, allowing for effective family planning, spacing of births, and reduction of unintended pregnancies. Hormonal contraceptives act by altering hormone levels in the body to prevent

pregnancy (Wright et al., 2020). In the evolving landscape of reproductive health, hormonal contraceptives represent a key option available worldwide (Holly et al., 2022). The most common types include oral contraceptives (often referred to as birth control pills), hormonal patches, vaginal rings, injections, and implants. Each method utilizes various combinations and dosages of estrogen and/or progestin to preventing ovulation, thickening cervical mucus, and thin the uterine lining, thereby reducing the likelihood of fertilization and implantation (Wright et al., 2020). Hormonal contraception is essential in reproductive healthcare, providing individuals the autonomy to plan their families and manage their reproductive health. By understanding the mechanisms, benefits, considerations, and emerging trends associated with hormonal contraception, individuals can make informed choices that suit their needs and preferences. However, the use of hormonal contraceptives may be associated with several side effects, such as headaches, weight gain, depression, nausea, breast tenderness, blood clots, stroke, heart attack, bleeding, and fatigue (Moumita and Monoj, 2022). Hematological profiles can serve as measurable indicators of blood, aiding in the identification and monitoring of certain pathological and physiological abnormalities (Esayas et al., 2022). Abnormalities in an individual's blood parameters can disrupt the homeostatic balance, potentially leading to various health conditions (Solomon et al., 2022).

While hormonal contraceptives are generally considered safe and effective, there is growing concern regarding their potential impact on biomarkers related to inflammation and oxidative stress within the body. Inflammation serves as a normal immune response to injury or infection; however, when it becomes chronic, it is linked to numerous health problems, including cardiovascular disease, diabetes, and cancer. On the other hand, oxidative stress occurs due to an imbalance between free radicals and antioxidants, leading to cellular damage. Both inflammation and oxidative stress play significant roles in the onset of various diseases (Morch et al., 2012). Reports have indicated a connection between hormonal contraceptives and an elevated risk of venous thrombosis, ischemic stroke, and heart attacks in certain women (Mukanga et al., 2023).

It is essential to comprehend the toxicological effects of hormonal contraceptives on inflammation and oxidative stress biomarkers to assess their overall safety. Monitoring these biomarkers in women using hormonal contraceptives could provide valuable insights into their long-term health consequences. Furthermore, understanding the specific mechanisms by which these contraceptives function may aid in the development of safer alternatives that minimize inflammatory and oxidative risks (Rosenberg et al., 2018). There is a notable lack of comprehensive reports, especially in low- and middle-income countries (LMICs), to substantiate claims that hormonal contraceptives negatively impact hematological profiles and biomarkers of oxidative stress and inflammation. Nevertheless, additional research is essential to investigate the effects of hormonal contraceptives on women's health. Therefore, more study is needed to assess the influence of hormonal contraceptives on hematological, antioxidant, and inflammatory parameters in users. This study would contribute to the body of knowledge surrounding the safety profile of hormonal contraceptives and aid in clinical decision making and patient counselling on choice of contraceptives.

MATERIALS AND METHODS

Study participants

For this study, a total of 50 participants were used; 25 of the total participants are women of reproductive age, using hormonal contraceptives, while the other 25 participants are women of reproductive age, not using hormonal contraceptives. The participants were recruited from Orita-Obele health center, Akure, Ondo State and Arakale health center, Akure, Ondo State.

Data collection

A data collection form (questionnaire) which contained items on demographic characteristics, anthropometric characteristics, duration of exposure, type of hormonal contraceptives used, common side effects experienced, diet/physical activity were used for subjects' recruitment. The data collection was all done by personal interview and informed consent form was obtained from all participants after educating them on the benefits and relevance of the study. Ethical approval was obtained from University of Medical Sciences, ethic committee.

Biochemical analysis

Blood samples were withdrawn from each subject by venipuncture. Blood samples collected were further analyzed.

Determination of hematological indices

The samples are analyzed using an autoanalyzer (Auto hematology analyzer GB LH5000-Gijuwie biomedical electronics). The collected blood samples were further analyzed for the following parameters

- Superoxide dismutase (SOD) activity
- TNF-alpha levels

Parameters were performed using Randox test kits using ELISA method of assay and according to the manufacturer's instructions.

Antioxidant activity

Superoxide dismutase (SOD) (Rotruck *et al.,* 1973). Measurement of SOD in each sample by ELISA kits is based on WST-8 method. The WST-8 method is based on the colorimetric reaction of WST-8. In the reaction, xanthine oxidase (XO) catalyzes the oxidation conversion of xanthine to yield superoxide anion, which quenches WST-8 to produce water-soluble formazan (a purple dye). As SOD quenches superoxide anion, thus its activity inhibits the overall colorimetric reaction. Therefore, the inhibition levels are used to indicate the SOD activities in cells, tissues or other biological samples.

Biomarker of inflammation

Tumor necrosis factor (TNF-α) (Markham *et al.,* 1995). The Human (TNF-α) ELISA employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TNF-α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF-α present is bound by the immobilized antibody. Unbound samples are removed during a wash step, and then a detection antibody specific for TNF-α is added to the wells and binds to the combination of capture antibody-TNF-α in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. A colored product is formed in proportion to the amount of TNF-α present in the sample.

RESULTS AND DISCUSSION

Statistical analysis

The data obtained were statistically analyzed with Shapiro-wilk test for normality using GraphPad prism 9.0) and the test of homogeneity of variance. An unpaired t-test was then performed to compare the means between the control and subjects using hormonal contraceptives. The results were presented as mean ± standard error of mean (SEM). Differences between means of case and control values at $p<0.001$ at 99.9% confidence interval for hematological parameters, TNF-α and SOD were considered significant (Figures 1-5).

This study evaluated the effects of hormonal contraceptives on hematological parameters. According to the results obtained from this study, a significant increase ($p<0.001$) in the level of WBC, granulocytes, and MID were observed in the case when compared to the control. White blood cells are a major part of the immune system, participating in both the innate and humoral immune responses (Figures 1-5). They circulate in the blood and mount inflammatory and cellular responses to injury or pathogens (Tigner *et al.,* 2022). In agreement with this study, Kovats (2015), Karolina *et al.,* (2019), reported increase in the number of neutrophils in the bloodstream as estrogen concentration increased. Estrogen, being one of the constituents of hormonal contraceptives, had been implicated in a number of pathological conditions involving the liver, bones, and muscles; nervous and cardiovascular systems; and in the immune system (Karolina *et al.,* 2019). Estrogen has been shown to modulate white blood cells (macrophage) function by regulating chemotaxis, cytokine production, phagocytosis, and Nitric oxide production (Okoroiwu *et al.,* 2021, Morgan *et al*., 2022). The observed elevated levels of white blood cells may be due to an increase inflammation induced by estrogen.

A significant increase ($p<0.05$) was also observed in the Platelet large cell ratio (PLCR) in the case when compared to the control. Increased percentage of large platelets suggest possible risk of thrombosis or cardiovascular complications (Karolina *et al.,* 2020). Thrombosis is the formation of blood clot which causes obstruction in the blood flow through circulatory system (Ayub *et al.,* 2016). However, estrogen has been shown to increase the risk of venous thrombosis, including deep vein thrombosis and pulmonary embolism (Abou-Ismail *et al.,* 2020). As reported by Corinne *et al.,* (2022), Thrombosis risk with estrogen-containing compounds increases with increasing systemic dose of estrogen. While progesterone-only–containing products are not associated with thrombosis, these components, along with patient-specific factors, may influence the choice of hormonal preparation.

However, there was no significant difference was observed ($p>0.05$) in the levels of red blood cell count (RBC) in case and control groups. This result is contrary to report by Coffie, *et al.,* (2020), who reported an increase in RBC and other related blood cells parameters among hormonal contraceptives users when compared to non-hormonal contraceptive users, this finding was attributed to the reduced menstrual flow among the subjects, which is known to be common among hormonal contraceptives users (Coffie, *et al.,* 2020).

A significant increase ($p<0.001$) was observed superoxide dismutase level (SOD) and a significant increase ($p<0.01$) was also observed in tumor necrosis factor alpha (TNF-α) levels in case when compared to control group. This is in agreement with a study by Quinn *et al.* (2022) who reported that blood biomarkers of oxidative stress in women using oral contraceptives (OC) during exercise showed significant elevations in malondialdehyde (MDA), a marker of lipid peroxidation, indicating increased oxidative stress among OC users compared to naturally cycling women that are not using contraceptives, (Zanotti *et al.* 2022), also reported that combined oral contraceptive users exhibited significantly increased oxidative stress markers, including hydroperoxides, with p-values indicating strong associations ($p < 0.001$). However, a contradicting report by Friedman *et al.* (2019), indicated that there is no significant difference ($p>0.05$) in oxidative stress markers among women using hormonal contraceptives compared to non-users. SOD plays a vital role in combating oxidative stress by catalyzing the dismutation of superoxide radicals into oxygen and hydrogen peroxide (Falade *et al*., 2014).

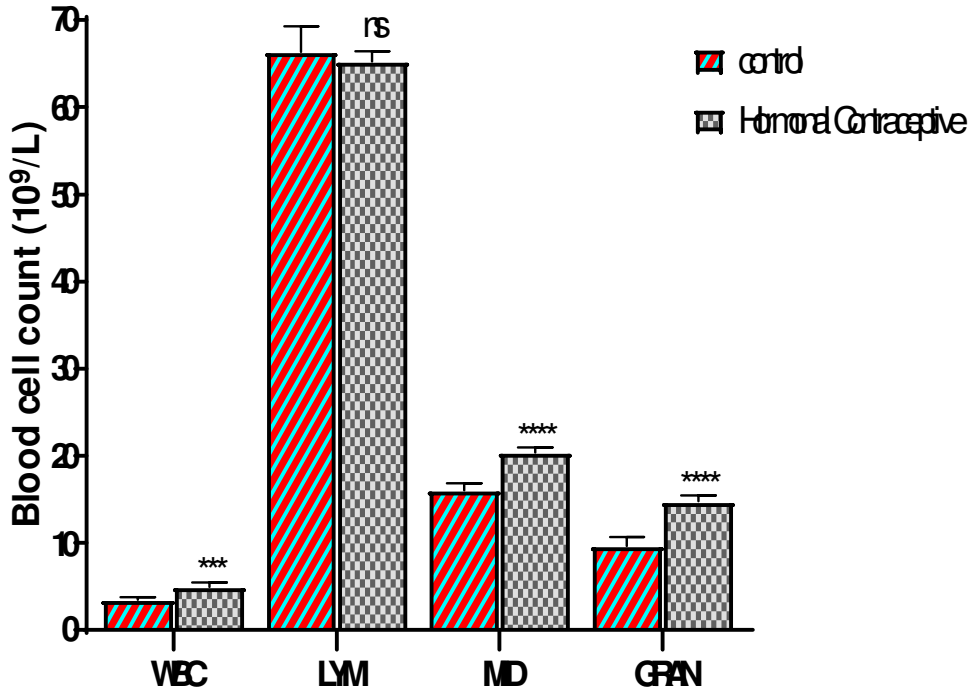

Figure 1: Effects of hormonal contraceptives on blood cell count: WBC- White blood cell, RBC-Red blood cell, MID-Mid-sized WBCs, LYM- Lymphocytes, GRAN-Granulocytes. ***p<0.001, ****p<0.0001 ^{ns} p>0.05; significantly different vs control. NS - not significant vs control.

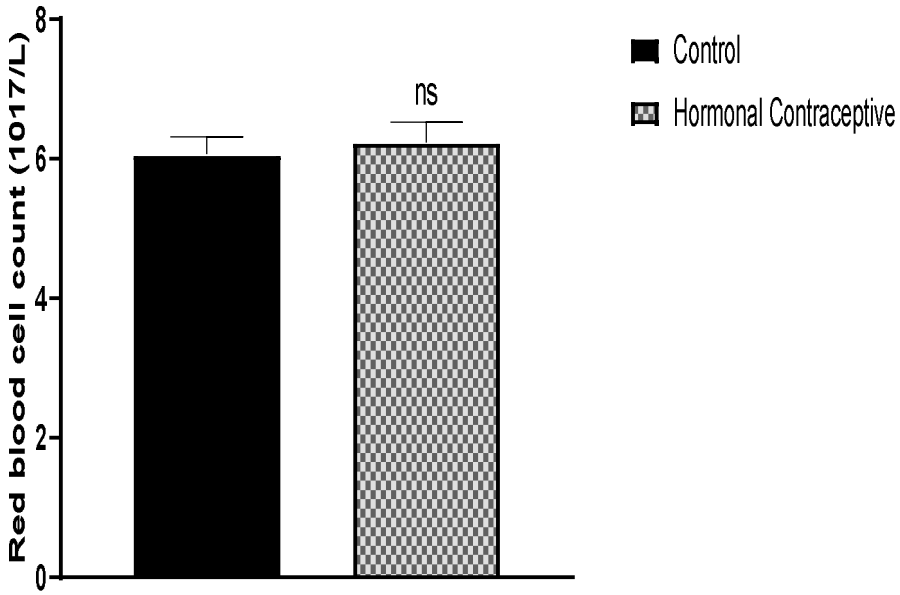

Figure 2: Effect of hormonal contraceptives on red blood cell count (RBC count). P>0.05 vs control. NS - not significant.

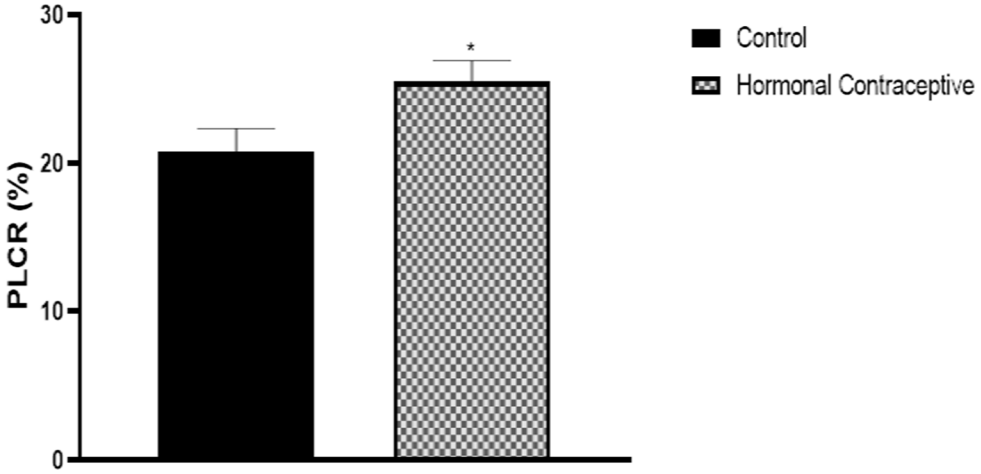

Figure 3: Effects of hormonal contraceptives on Platelet Large Cell Ratio (PLCR). Bars represent Mean ± Standard Error of Mean (SEM), (n = 20 patients/group). *p<0.05; significantly different vs control.

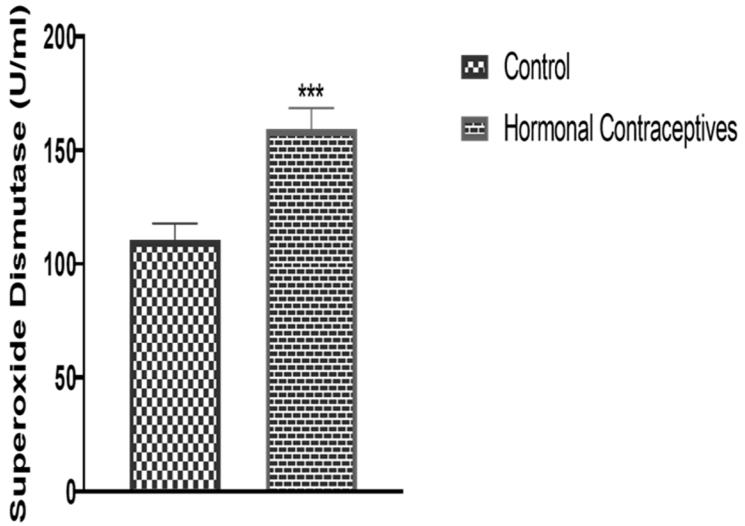

Figure 4: Shows the concentration of SOD of Control and subjects using Hormonal contraceptives **p<0.001; significantly different vs control.

The decrease or increase in SOD activity can reflect the balance between oxidative stress and antioxidant defense mechanisms in the body (Brassfield *et al.,* 2014). Imbalance in SOD activity can induce oxidative stress, which can lead to cellular damage and contribute to various diseases such as chronic and degenerative ailments like cancer, arthritis, aging, autoimmune disorders, cardiovascular and neurodegenerative diseases (Falade *et al.,* 2014).

The significant increase observed in TNF-α (a proinflammatory factor) from this study (p<0.01) is consistent with several other studies, Genazzani *et al.,* 2017 reported significant increase in inflammatory markers (CRP, TNF-α, and interleukin-6) in subjects on combined estrogen-progestin contraceptives, Sánchez-Borrego *et al.* (2020), reported that desogestrel significantly increased levels of CRP and TNF-α , Katz *et al.,* (2023), Mengelkoch *et al.,* (2023) reported that women on HC had significantly higher levels of TNF-α both before and after stress tests, indicating a pro-inflammatory response associated with

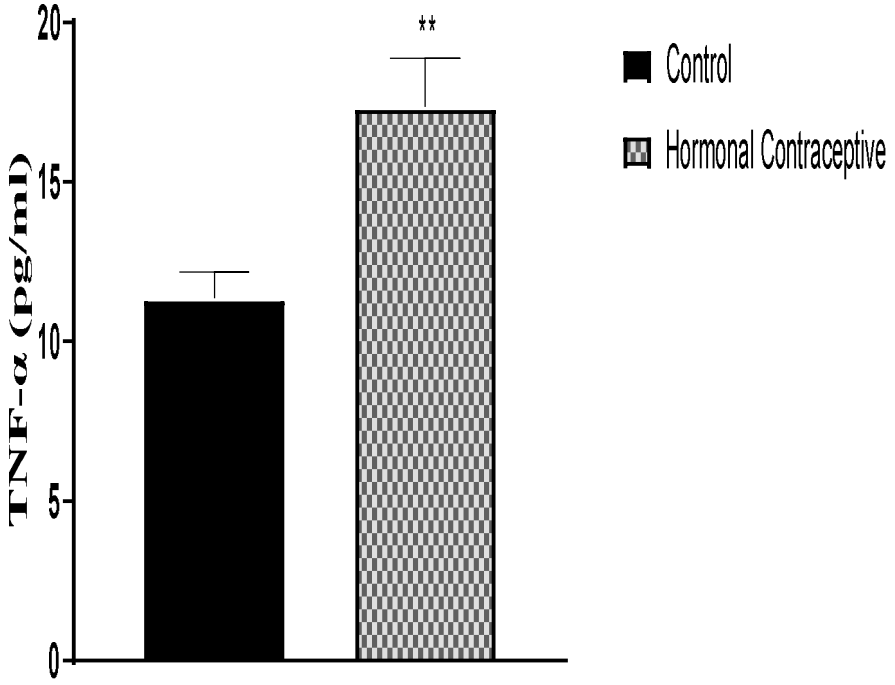

Figure 5: concentration of TNF-alpha of control and subjects using Hormonal contraceptives. **p<0.001; significantly different vs control.

HC use, also Ramzi *et al.,* (2024) observed that long-term use of oral contraceptives is linked to low-grade chronic inflammation and elevated C-reactive protein (CRP) levels in women, suggesting an inflammatory response due to hormonal contraceptive use. Hormonal contraceptives may induce a state of chronic inflammation by increasing the levels of inflammatory markers like CRP, TNF-α, and interleukin-6. Changes in TNF- α levels observed from this study highlights a potential link between hormonal contraceptives and inflammatory responses.

Declarations

Funding: Not applicable

Consent to participate: Not applicable.

Ethics approval: was given by the quality control unit of the University of Medical Sciences, Ondo, Nigeria.

Code availability: Not applicable

Competing interest: The authors declare that there are no conflicts of interest.

Availability of Data and Material: Data generated as part of this study is available upon request from the corresponding author.

Consent for Publication: All authors provide consent for publication.

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