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Effect of oral contraceptive pills on oxidative stress in Saudi women

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Abstract

Objective The aim of this study was to evaluate the effect of oral contraceptive pills (OCPs) on oxidative stress in Saudi women.

Methods A total of 55 Saudi women were divided into two groups: users of OCP (N=30) for at least 1 year and non-users (NOCP, N=25). The demographic data were obtained through face-to-face interviews performed by the researcher. Blood specimen from both groups were drawn after 8 h of fasting to estimate serum total antioxidant (TAOC), nitric oxide (NO), C-reactive protein (CRP), vitamins (E, B6, B12), and some hematological parameters: hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC).

Results The results showed a significant decreased in serum TAOC, NO, vitamins E, and B6 accompanied with high significant increase in CRP level while no significant changes B12, HB, RBC, and WBC were observed in the OCP users as compared to the control group.

Conclusions The results of this study indicated that the use of OCP resulted in low levels of the total antioxidant, nitric oxide, vitamins E, and B6 with a significant increase in CRP. Women who used OCP may be more susceptible to oxidative stress by enhanced depletion of antioxidants.

Introduction

Uncontrolled pregnancy is one of the most important challenges of the century.[1,2] Oral contraceptive pills (OCP) continued to be one of the most common methods of contraception used by the majority of women. Across all ages, women in their 20s are the most likely to use OCP.[3]

The OCP is the commonest contraceptive method used among Saudi women.[4] Combined pills and progestin-only pills are the two types of OCP.[5] The usage of OCP has been shown to cause oxidative stress by enhanced depletion of antioxidant and increased lipid peroxidation.[6]

Oxidative stress is defined as an imbalance between antioxidants and pro-oxidants in the cells, which are manifested by high levels of free radicals.[7] Free radicals are deleterious to the human body because they can retrieve electrons from various molecules provoking the formation of oxidized forms so that severe oxidative stress can even trigger cell apoptosis and necrosis.[8]

Antioxidants systems, both non-enzymatic and enzymatic, are produced in the body to block too much free radicals production.[9] Non-enzymatic antioxidants are known as synthetic antioxidants or dietary supplements, including vitamin C, vitamin E, β-carotene, and so on.[10] Mounting evidence suggests that oxidative stress could play a pivotal role in the pathogenesis of several diseases including inflammatory, muscular, cardiovascular, and neurodegenerative diseases.[11]

Nitric oxide (NO) is a key organizer of the endothelia functional in the cardiovascular system. Change of the redox equilibrium in the vascular system intervenes with NO product and modifies vascular homeostasis bioavailability, promoting the development of metabolic and cardiovascular diseases.[12] Estrogen generally has diverse vascular actions via increasing the bioavailability of NO on activation of NO synthase, and NO is inversely associated with oxidative stress.[13] Lobysheva et al.[14] observed a correlation between the use of combined oral contraceptives pills (COCP) and increased oxidative stress and the decreased NO in women who used OCP. Hassan et al.[15] reported that NO is significantly reduced in COCP users than nonusers.

Vitamins also immediately scavenged ROS and upregulated the activity of antioxidant enzymes. Vitamin E is considered one of the most vital antioxidants; vitamin E prevent peroxyl radicals generated by peroxidation of polyunsaturated fatty as a result of ROS, therefore, protecting cells against oxidation of membrane phospholipids.[16] Hassan et al.[15] concluded that serum vitamin E levels are significantly lower among the OCP users' groups. Vitamins B6 and B12 play crucial inter-related roles in DNA synthesis throughout the lifecycle, especially during childhood, adolescence, and the reproductive years for women.[17] Low vitamin B6 status has been associated with an increased risk of cardiovascular disease.[18] The use of OCP causes many biochemical changes in; clotting factors, thrombosis, platelet changes, atherosclerosis, and inflammatory profiles.[19-21] Jamil et al.[22] concluded that hemoglobin levels are normal in the OCP users. Baker et al. (2016) reported that the WBC count elevates in the woman using OCP due to that the OCP may cause some internal infection in the women.

CRP is generated via the liver, and CRP levels rise within the presence of any inflammatory in the body.[23] Serum levels of CRP are often used to detect inflammatory signs to estimate cardiovascular sickness and stroke.[24] Ferreira et al. (2017) found a significant increase in CRP levels, especially among overweight women who use COCP more than 3 mg/dL (a boundary value related to the evolution of cardiovascular diseases). This study aimed to evaluate the effect of OCP on oxidative stress in Saudi women.

Materials and Methods

The study was carried out on 55 Saudi women were divided into two groups: women taking an OCP (N=30) and women who had never taken the OCP (N=25). The ethical approval was obtained through the local ethics committee at the King

Abdul-Aziz University Hospital. All relevant information was recorded on predesign questionnaires. A blood specimen (6 ml) was collected by venipuncture after 8–12 h of fasting were collected to estimate various parameters: total antioxidants, NO, vitamins E, B6, and B12 by colorimetric method using ELISA Kit. Hb, RBC, and WBC were estimated using a hematology analyzer. The CRP level was also determined by a commercial Kit from SIMENS.

Statistical Package for Social Science (SPSS) computer software was used for data analysis. Means ± standard deviation (SD) was calculated. Independent *t*-test and Spearman's correlation were used to analyze the differences between bath OCP users and non-user control. The results were considered statistically significant at $p \leq 0.05$.

Results

The current results showed that total antioxidant and nitric oxide were significantly decreased (p=0.000) among women using oral contraceptive when compared to control group (Table 1).

As shown in Table 2, there was a highly significant (p=0.000) reduction in the vitamins E and B6 in OCP users compared to the control group. However, there was no significant difference (p=0.32) in vitamin B12 between the cases and control.

Data demonstrated in Table 3 showed that OCP induced a non-significant changes in Hb, RBC, and WBC (p=0.06, 0.52, 0.90 respectively), associated with a highly significant increase (p=0.000) in CRP, compared to NOC.

From Table 4, it could be observed that there was a significant changes association between duration of OCP use and TAOC, NO, CRP, vitamins E, and B6. Moreover, Hb was significantly higher started after 5 years, while, vitamin B12 and WBC were significantly different started 3–4 years. No significant differences in the RBC were noticed along with the tested duration (1–7 years) (Table 4).

Table 1. **The effect of OCP on Total antioxidant and NO.**

Variables	Group	N	Mean	p-value	Sig
TAOC	NOC	25	21.82	0.000	H. Sig
	OCP	30	4.89		
NO	NOC	25		0.000	H. Sig
	OCP	30	22.05		

NOC non-oral contraceptives, OCP oral contraceptives pills, TAOC total antioxidant, NO nitric oxide.
Values are as Mean±SD, p<0.05 significant as compared to the non-users.

Table 2. **The effect of OCP on vitamins.**

Variables	Group	N	Mean	p-value	Sig
Vitamin E	NOC	25		0.000	H. Sig
	OCP	30	24.379.87		
Vitamin B6	NOC	25	627.65123.38	0.000	H. Sig
	OCP	30	274.1870.24		
Vitamin B12	NOC	25	267.4867.05	0.32	N. Sig
	OCs	30	248.46		

NOC non-oral contraceptives, OCP oral contraceptives pills.
Values are as Mean±SD, p<0.05 significant as compared to the non-users.

Table 3. **The effects of OCP on Some Hematological Parameters and CRP.**

Variables	Group	N	Mean	p-value	Sig
Hb	NOC	25		0.06	N. Sig
	OCP	30	12.16 1.77		
RBC	NOC	25	4.32 0.58	0.52	N. Sig
	OCP	30	4.23 0.42		
WBC	NOC	25	6.99 1.73	0.90	N. Sig
	OCP	30	7.08 3.59		
CRP	NOC	25	0.16	0.000	H. Sig
	OCP	30			

NOC non-oral contraceptives, OCP oral contraceptives pills, HB haemoglobin, RBC red blood cells, WBC white blood cells, CRP C-reactive protein.
Values are as Mean±SD, p<0.05 significant as compared to the non-users.

Discussion

Oral contraceptive steroids are one of the factors that enhance oxidative stress and lead to the formation of free radicals. Oxidative stress constitutes a disturbance caused by an imbalance between the generation of free radicals and the antioxidant system, which causes damage to biomolecules. This, in turn, may lead to the occurrence of many chronic degenerative diseases.[25]

The free radical-mediated peroxidation of membrane lipids increases permeability and membrane fluidness with loss of its integrity that leads to cell damage.[26] Antioxidants work cooperatively in biological systems, and it is important to be able to correlate antioxidant measurements with antioxidant defenses and disease prevention. It is therefore recommended to study "total antioxidant capacity", rather than monitoring individual antioxidant levels, which may be less affected by dietary habits.[27] In this study, the total antioxidant level was significantly decreased among women using OCP. This result was in agreement with Palan et al. (2010) and Adejumo et al.[28] The behavior of molecules related to oxidative stress can differ according to types and doses of estrogen, progestogen, or the particular compounds of estrogen and progestogen.[29]

Estrogens display an antioxidant activity by inhibiting the expression and function of the NADP+/NADPH oxidase,[30] by increasing the expression and level of activation of the endothelial isoform of the nitric oxide synthase (eNOS)[31] and by stimulating the expression and activity of the manganese SOD (MnSOD) and of the extracellular SOD (ecSOD).[32] These antioxidant activities of estrogens are counteracted by progestins via the activation of the NADPH oxidase and the inhibition of the expression and activity of the MnSOD and of the ecSOD.[33] Therefore it implies that the counteractive effect of progestin would result in a decrease in the serum total antioxidant status especially, in women taking either COCP.[28]

In the current study, there is a significant decrease in serum NO in the OCP users as compared to the control group.

Table 4. **Effect of duration of OCP use on biochemical parameters.**

	Control N = 25	Duration of OCs		
		1-2 years n = 7	3-4 years n = 10	5-7 years n = 13
TAOC	21.828 ± 5.674	5.357 ± 1.651 P= 0.000*	4.860 ± 1.706 P= 0.000*	4.669 ± 2.200 P= 0.000*
NO	171.316± 34.8	45.771 ± 21.229 P= 0.000*	41.220 ± 25.322 P=0.000*	40.892 ±21.422 P= 0.000*
CRP	0.160 ± 0.071	1.614 ± 0.467 P= 0.000*	1.440 ± 0.384 P=0.000*	1.608 ± 0.528 P=0.000*
Vit E	96.524±25.026	23.843 ±11.652 P= 0.000*	22.460 ± 8.921 P=0.000*	26.139 ± 10.081 P= 0.000*
Vit B6	627.652±123.389	284.343 ± 69.892 P= 0.000*	289.060 ± 92.414 P= 0.000*	257.262 ± 50.212 P= 0.000*
Vit B12	267.480 ± 67.053	278.00 ± 50.471 P= 0.523	224.300 ± 70.539 P=0 .049*	251.154 ± 85.517 P= 0.508
HB	11.204 ± 2.054	11.450 ± 2.441 P= 0.600	12.019 ± 1.125 P= 0.547	12.657 ± 1.760 P= 0.047*
RBC	4.321 ± 0.588	4.134 ± 0.360 P= 0.218	4.234 ± 0.260 P=0.371	4.289 ± 0.555 P= 0.712
WBC	7.156 ± 1.604	6.370 ± 1.382 P= 0.210	5.729 ± 0.811 P=0.008*	6.869 ± 1.673 P= 0.470

The values are the mean ± S.D. of parameters measured.
Significantly different from control value at p<0.05*, 0.01**, 0.001***

A similar result was reported by Lobysheva et al.[14] Hassan et al.[15] observed that taking COCP resulted in a significant decrease in NO level. Estrogen is generally has diverse vascular actions via increasing the bioavailability of NO on activation of NO synthase, and NO is inversely associated with oxidative stress.[13] Decreased bioavailability of NO may result in abnormal reactions between the vessel wall and platelets and is thus involved in the initiation and progression of atherosclerosis.[34]

The present study reported a significant decrease in vitamins E, B6 and no significant change in B12 in the OCP users as compared to the control group. A similar result was reported by Palan et al. (2010), Wilson et al. (2011) and Singh et al. (2018). The study by Hassan et al.,[15] observed a decrease in the serum vitamin E, especially in women taking either COCP. In contrast to our results, Mcarthur et al. (2013) and Berenson and Rahman,[35] have reported that the young women using OCP had significantly lower serum vitamin B12 concentrations.

Vitamin E level was significantly decreased among women using OCP. The mechanism of such an effect is unknown, but it was thought that, at its high level, certain estrogens, in particular diethylstilbestrol, may produce reactive oxygen species that may attack a number of biological substances, including lipids and DNA, and could natural antioxidants may be consumed to trap such compounds.[36] The OCP may have some relatively specific effect on tryptophan's metabolism, which is independent of vitamin B6 levels. This effect may be primarily on the activity of tryptophan oxygenase since studies in rats have shown directly as well as adrenal-mediated effects of estrogens on the activity of this enzyme.[37] Previous studies[38,39] indicated that steroids or steroid conjugates could affect the activity of the PLP-dependent enzymes of the kynurenine pathway, i.e., kynureninase and kynurenine aminotransferase. Thus, it seems most likely that the altered tryptophan metabolism of oral contraceptive users is the summation of hormonal effects on tryptophan oxygenase and kynureninase rather than the production of a general vitamin B6 deficiency.[40,41] Vitamin B12 level was no significant difference between OCP users and nonusers because that absorption is not affected and that redistribution of B12 throughout the body could be responsible.[42]

In this study, level Hb and WBC were showed a non-significant decrease in non-oral contraceptive users. Regarding hematological parameters, different results were observed by several researchers. Toryila et al.[43] have reported a reduction in Hb, RBC, WBC among women using COCP compared to the controls. Jamil et al.[22] showed Hb levels are normal in the OCP. Al-Zayadi[44] and Yeasmin et al.[45] have reported increases in Hb level comparing with control groups. Toryila et al.[46] observed an increase in WBC count in COC-treated female Wistar rats. The difference may be as a result of the use of different COC with different concentrations of estrogen and progesterone and the duration of use. Mooij et al. (1992) reported no significant difference on hematological parameters due to OCP use in women, but serum iron status was significantly increased for the users of OCP.

In this study a no significant change in RBC among women using OCP. A similar result was reported by Al-Zayadi.[44] Due to the confounding of the effects of dose and the type of hormone, and the formulation of the drug, it was not easy to estimate the effect of OCP on RBC. Even though the combination of estrogen and progesterone would be considered as advance formal of the OCP, the use of it still strongly associated with vascular disease.[47]

The results indicated a significant increase in CRP in the OCP users as compared to the control group; this finding is in agreement with Divani et al.[48], Ferreira et al., (2017), Guedes et al.[49], and Oliveira et al.[50] It was indicating that OCP leads to an increased subclinical inflammatory process. Divani et al.[51] established that progestin increases interleukin 6 (IL-6, an inflammatory-mediated stimulation) of CRP in combination with conjugated equine estrogen and showed that IL-6 change was negatively linked to CRP change when subjects were treated only with equine estrogen. The involvement of progestin has been suggested to induce IL-6-mediated CRP stimulation, whereas other mechanisms are likely to be responsible for the development of CRP in women receiving only conjugated equine estrogen.[52]

This study evaluates the effects of duration of OCP use on oxidative stress, which is represented by a significant decrease in TAOC, NO, vitamins E, and B6 levels. In contrast, CRP has shown a significant increase. Egoro *et al.* (2018) demonstrated that elevated levels of plasma CRP in long-term users of OCs containing lower doses of estrogen and progestin composition for ≥2 years as compared to (control group). However, it was observed a significant change in Hb, WBC, and B12 after 3 years of using OCP. Similarly, Toryila et al.[43] reported that long- term use of COCP might lead to more complications than short- time use. However, the long-term users of the OCP on RBC has shown no significant difference ($p \geq 0.05$) compared to the mean value of non-users of OCs (control group).

Conclusion

The results of this study indicated that the use of OCP resulted in low levels of total antioxidant, NO, vitamins E, and B6 with a significant increase in CRP. Moreover, there is a statistically significant relationship between OCP use and increased oxidative stress.

Conflict of Interest

None

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