Long Term Oral Contraceptive Administration is Associated with Low Serum Levels of Nitric Oxide, Vitamin C and Vitamin E

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Abstract

Background: Oral contraceptives are now commonly used by women worldwide as a method for preventing pregnancy. In addition, it was proposed that women taking these drugs require supplementary vitamin E. The aim of this study was to identify changes in serum levels of nitric oxide, vitamin C and vitamin Einwomen taking oral contraceptives for more than three years.

Method: The study included (60) women using these tablets and (58) healthy women used as control group. Levels of nitric oxide, vitamin C, vitamin E were estimated in all women participants. In order to estimate the effect of age on levels of nitric oxide, vitamin C and vitamin E, women were divided into two groups: thirteen women aged (18-35) years and thirteen of women aged (36-45) year, remarked to it with symbols (A and B). These groups were compared with two groups of nonuser's women: twenty-nine of women aged (18-35) years and twenty-nine of women aged (36-45) year, remarked to it with symbols (AC and BC).

Results: There was a significant decrease in levels of nitric oxide, vitamin C and vitamin E) ($P \leq 0.05$) in all women who were taking oral contraceptives when compared with control women. In addition, the results illustrated significant elevation in nitric oxide,vitamin C and (vitamin Elevels ($P \leq 0.05$) in the two groups of user's women when compared with the control groups. Also, it showed none significant elevation in nitric oxide in group (B) in comparison with group (A) ($P \leq 0.05$), while no significant differences were seen in vitamin C and vitamin E levels ($P \leq 0.05$) between groups (A) and (B) and control groups.

Conclusion: The results of this study indicated that the use of combined oral contraceptives resulted in low levels of nitric oxide, vitamin C and vitamin E.

Keywords: *Oral contraceptives, nitric oxide, vitamin C, vitamin E.*

Introduction

Oral contraceptives (OCs) are now commonly used by millions of women worldwide as a method for preventing pregnancy[(1)]. The use of present contraceptives has contributed substantially to the reduction of maternal and infant morbidity and mortality and to the ability of women to contribute to society[(2)]. There are three types of oral contraceptive pills: combined estrogen-progesterone, progesterone only and the continuous or extended-use pills. The most commonly prescribed pill is the combined hormonal pill with estrogen and progesterone. Progesterone is the hormone that prevents pregnancy, while the estrogen component will control menstrual bleeding. Birth control pills are primarily used to prevent pregnancy[(3)]. Some serious side effects have been reported in women taking them. Studies have indicated a relationship between

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oral contraceptives use and cardiovascular disease [4]. Oral contraceptive steroids are one of the factors that enhance oxidative stress and lead to the formation of free radicals. Oxidative stress (OS) constitutes a disturbance caused by an imbalance between the generation of free radicals and antioxidant system, which causes damage to biomolecules. This, in turn, may lead to the occurrence of many chronic degenerative diseases[5]. Reactive oxygen species (ROS) or 'free radicals' are highly reactive oxygen-derived molecules characterized by having unpaired electrons in their outer valence orbital. They include oxygen-centered radicals (hydroxyl radical, nitric oxide radicals and superoxide anion radical[6]. NO• is generated in biological tissues by specific NO synthase that metabolizes arginine to citrulline via five-electron oxidative reaction. At a physiological pH of 7.2, the sodium nitroprusside compound is decomposed into aqueous solution and generates NO•. Stable products (nitrate and nitrite) are produced when NO• reacts with oxygen under aerobic conditions, which can be determined by using the Griess reagent [7]. The antioxidant activity of estrogen has been attributed to prevention of expression and function of $NADP^+$/NADPH oxidase, an increase in expression and activation level of endothelial isoform of nitric oxide synthase (eNOS) and stimulation of the expression and activation of manganese superoxide dismutase[8]. Exogenous estrogen administration restores endothelial function by enhancing nitric oxide synthesis through genomic and non-genomic mechanisms and by reducing oxidative stress and nitric oxide breakdown[9]. Antioxidants are free radical scavengers which help in delaying or prevention oxidation by trapping free radicals. The normal vital concentration of free radicals or reactive oxygen species (ROS) in living organisms is maintained by antioxidants[10]. Well-documented dietary antioxidants include ascorbic acid (vitamin C), α-tocopherol (vitamin E), polyphenols and carotenoids[11]. In line with preclinical findings, Briggs and Briggs[12] showed that combined-type OCs decreased plasma tocopherol in healthy Caucasian women and, therefore, proposed that women taking these drugs require supplementary vitamin E. In line with this hypothesis, it has been stated that vitamin C levels in platelets and leukocytes are lowered by use of OCs, specifically those containing estrogen, which is thought to increase the rate of metabolism of vitamin C. Other authors reported that with adequate dietary intake of ascorbic acid, there is no threat on ascorbic acid status as a result of using OCs[12].

Subjects and Method

The study was carried out on 60 women taking oral contraceptives and fifty-eight healthy age-matched women (control group). Users of oral contraceptives had attended the family planning clinic at Maternity and Pediatric Hospital in Nassyrieh, Iraq. The age of users and controls ranged from (18-35) year. According to age, women used combined oral contraceptives were divided into two subgroups;Group A included thirteen women aged (18-35) year and group B included thirteen women aged (36-45) year. Also, the women who were not using combined oral contraceptives were divided into two subgroups similar to the users in the age Group AC included twenty-nine women and Group AB included twenty-nine women.

Blood samples were obtained from user women and control group by venipuncture. Samples were allowed to clot at 37°C and then centrifuged at 3000xg for 10min. Sera were removed and stored at (-20°C) for later measurement of biochemical parameters, unless used immediately.

Determination of Serum Nitric Oxide Levels: The method described by[13] has been used to estimate the concentration of nitric oxides (NO). The principle of this method included measurement of nitrite oxide (NO-2), which is the most stable oxide of nitrogen oxides and the addition of zinc sulphate to serum sample which works on the deposition of proteins first and reduction of nitrate oxide (NO-3) to nitrite oxide (NO-2). Nitrite oxide, in acidic medium, turns into nitrous acid (HNO2) which nitratesthe sulfanyl amide to give the dizonium salt sulfanyl amide diazonium. The latter, in turn, condenses with the alpha-naphthylethylene diamine dihydrochloride complex whose absorption is measured at wavelength 540nm.

Determination of serum vitamin C concentrations: This method was based on the principle that ascorbic acid is oxidized by copper to form dehydroascorbic acid and diketogulonic acid. These products are treated with 2,4-dinitrophenyl hydrazine (DNPH) to form the derivative bis-2,4-dinitrophenyl hydrazone. This compound in strong sulfuric acid undergoes rearrangement to form a colored product which is measured at 520nm. The reaction was run in the presence of thiourea to provide a mildly reducing medium, which helps prevent interference from non-ascorbic acid chromogens[14].

Determination of serum vitamin Econcentration: In this method determination of tocopherol in serum was done by colorimetric method. Serum vitamin E was measured by the method of Baker on the basis of reduction of ferric ions to ferrous ions by vitamin E and the formation of complex with 2-2` dipyridyl and it was measured at 520nm[15,16].

Result and Discussion

Table (1) showed a significant decrease in the concentration of serum nitric oxide (NO) in the age groups (A) and (B) in comparison with their control groups (AC) and (BC) ($P \leq 0.05$). In addition, we found non-significant differences in the concentration of serum NO in group (B) in comparison with group (A). Also, there were non-significant differences between control groups (AC) and (BC).

The study indicated that the use of oral contraceptive pills resulted in low levels of nitric oxide. These results were different from those reported in a previous study [17]who found that taking low-dose oral contraceptives in healthy women did not causeany differences in levels of amino acid and nitric oxide, however, the results of this study were consistent with another previous study[18]. Estrogen can increase the bioavailability of nitric oxide (NO) in the endothelium, possibly by preventing the initiation of atherosclerosis [19]. The predominant isoform of NOS in the endometrium is eNOS and this is predominantly localized in the epithelial cell layer. Estrogen through both genomic and non-genomic mechanisms up-regulates eNOS and iNOS and phosphorylatese NOS in primary endometrial epithelial cells [20]. The inclusion of progestin in postmenopausal HRT appears to blunt the effects of estrogen on endothelial NO production [21]. It was found that the addition of medroxy progesterone acetate reduces the effect of estrogen on endothelium dependent relaxation[22]. OCT can increase oxidative stress, possibly leading to vascular complications[19]. Furthermore, progesterone may reduce ROS formation and cause vascular relaxation in a tissue-specific fashion[23]. However, progesterone antagonizes the vasoprotective effects of estrogen on anti-oxidant enzyme expression and function and enhances NADPH oxidase activity and the production of ROS[24]. 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. Estrogens are generally known to have various vascular actions by increasing the bioavailability of NO via activation of NO synthase[25].

Data in Table (2) showed a significant decrease in serum Vitamin C levels in groups (A) and (B) in comparison with control groups (AC) and (BC), respectively ($P \leq 0.05$) and there were no significant differences in serum vitamin C between groups (A) and (B) ($P \leq 0.05$). There were no significant differences between control groups (AC) and (BC). The results of this study were consistent with those reported by[26] who found that the key nutrients affected by oral contraceptives were folic acid, vitamins (B2, B6 and B12) and vitamins (C, A and E) as well as some minerals. Combined Oral Contraceptives also resulted in biochemical changes such as altered nutritional status with regard to several vitamins such as vitamin C [27]. Meanwhile, some of these micronutrients are cofactors and/or coenzymes of other enzymes and are involved in important metabolic pathways [28].

The decrease in the levels of vitamin C in postmenopausal females might be due to its increased consumption to counteract the increased oxidative stress and to inhibit membrane lipid peroxidation. Also, may be because vitamin C can restore the antioxidant properties of oxidized vitamin E, suggesting that a main function of vitamin C is to recycle vitamin E radical [29].

Data in Table (3) showed a significant decrease in serum Vitamin E levels in groups (A) and (B) in comparison with control groups (AC) and (BC), respectively ($P \leq 0.05$). Also, there were non-significant differences in serum vitamin E between groups (A) and (B) ($P \leq 0.05$). There were non-significant differences between control groups (AC) and (BC). Several review studies have shown that vitamin E and vitamin C were lower in OC users in comparison with non-users[30].

Table (1): Serum Nitric Oxide (NO) levels in oral contraceptive users and their controls according to their age groups

Age Group/yr	Users		Controls		*P*-value
	Subgroup (No.)	NO (µmol/mL) Mean±SD	Subgroup (No.)	NO (µmol/mL) Mean±SD	
18-35	A (30)	9.28±1.10*a	AC (29)	12.29±1.09**a	0.000
36-45	B (30)	8.90±1.21*a	BC (29)	12.30±0.95**a	0.000
P-value	0.17		0.97		

(*): was considered significantly different in comparison with control.([a]): were considered significantly different among groups of users.

Table (2) Serum Vitamin C levels in oral contraceptive users and their controls according to their age groups

Age Group/yr	Users		Non users		*P*-value
	Subgroup (No.)	Vitamin C(mg\dl) Mean±SD	Subgroup (No.)	Vitamin C(mg\dl) Mean± SD	
18-35	A (30)	0.20±0.08*a	AC (29)	0.45±0.07**a	0.000
36-45	B (30)	0.19±0.08*a	BC (29)	0.49±0.13**a	0.000
P-value	0.685		0.122		

(*): was considered significantly different in comparison with control.([a]): were considered significantly different among groups of users.

Table (3) Serum Vitamin E levels in oral contraceptive users and their controls according to their age groups

Age Groups/yr	Users		Controls		*P*-value
	Subgroup (No.)	Vitamin E(mg\dl) Mean±SD	Subgroup (No.)	VitaminE(mg\dl) Mean±SD	
18-35	A (30)	8.07±1.27*a	AC (29)	12.52±1.90**a	0.000
36-45	B (30)	7.28±1.98*a	BC (29)	12.28±2.58**a	0.000
P-value	0.128		0.647		

(*): was considered significantly different in comparison with control.([a]): were considered significantly different among groups of users.

Conclusion

The results of this study indicated that the use of combined oral contraceptives resulted in low levels of nitric oxide, vitamin C and vitamin E.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

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