

Effect of hormonal contraceptives on serum ceruloplasmin, copper, and Vit.C levels in Tikrit city

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Abstract

Oral contraceptive steroids are used by an estimated 60 to 70 million women worldwide. Over the past 20 years there have been both case reports and clinical studies on the topic of drug interactions with these agents. The use of oral contraceptives has been consistently associated with decreased levels of the activity of many enzymes, and Vitamin C. The main transporter of copper, α_2 -globulin ceruloplasmin which is a multifunctional enzyme. The present study was conducted in Family planning in Tikrit Teaching Hospital in Tikrit province, Serum ceruloplasmin, copper, vitamin C, and iron levels were estimated in 40 women who were using oral contraceptives for at least 1 year and 40 women who were using copper T (Intrauterine contraceptive device). The mean age of women was (33.08 years). Forty non- contraceptive users participated in the study as a control group. They were drawn from the same population and matched for age with the contraceptive-users group. The serum level of ceruloplasmin (Cp) as antioxidant protein was significantly higher in women were using oral contraceptive than control group (60 ± 0.16 mg/dl vs. 36.5 ± 0.96 mg/dl, $P < 0.05$) while the difference between the serum (Cp) in control and women using copper T is statically non significant $P < 0.05$, also mean of serum copper in women were using contraceptive (18 ± 0.612 μ M/L) is significantly higher ($P < 0.01$) than the mean of control (11.2 ± 0.474 μ M/L), while the difference between the serum copper in control and women using copper T is statically non significant ($P < 0.01$). On the other hand the serum vitamin C values in women using oral contraceptives and copper T (5.57 ± 0.02 , 6 ± 0.031 μ M/L respectively) were significantly lower than control group (10.73 ± 0.09 μ M/L, $P < 0.05$).

تأثير موانع الحمل الهرمونية على مصل سيريلوبلازمين والنحاس وفيتامين C في مدينة تكريت

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المستخلص

يبلغ عدد النساء اللواتي يتعاطين موانع الحمل من 60-70 مليون، وأجريت دراسات عديدة عن مدى الخطورة الناتجة من استعمال هذه الأدوية، كما وردت تقارير عن تأثير هذه الأدوية في خفض فعالية بعض الأنزيمات كالسيريلوبلازمين (وهو عبارة عن ألفا كلبولين وهو مركب رئيسي في نقل النحاس)، وفيتامين C. أجريت الدراسة الحالية في وحدة تنظيم العائلة في مستشفى تكريت العام. إذ شملت الدراسة 40 امرأة يستعملن حبوب منع الحمل (مايكروجينون-ليفونورجستريل) (0.15 mg) واثنيل ابيسترايول (0.03 mg) او يستعملن اللولب عددهن 40 إذ بلغ معدل أعمارهن 33.08 سنة. تم اعتماد (40) امرأة سليمة لا يتعاطين موانع الحمل كمجموعة ضبط تم اختيارهن من نفس الشريحة السكانية وكن متوافقات عمرياً مع المجموعة المستخدمة لموانع الحمل تم جمع عينات الدم من المجموعتين لغرض تقدير فعالية سيريلوبلازمين والنحاس والحديد وفيتامين C في المصل. أظهرت نتائج الدراسة الحالية ارتفاع معدل مستوى السيريلوبلازمين والنحاس والحديد وفيتامين C في مصل

النساء اللاتي يستعملن حبوب منع الحمل 60 ± 0.16 mg/d بالمقارنة مع المجموعة الضابطة 36.5 ± 0.96 mg/dI، بينما لم تتأثر مستوى السيريلوبلازمين في مصل النساء اللاتي يستعملن اللولب (37.29 ± 0.99) بالمقارنة مع المجموعة الضابطة 36.5 ± 0.96 mg/dI، كما ارتفع مستوى النحاس بشكل معنوي $(P < 0.05)$ ، 36.5 ± 0.96 mg/dI بالمقارنة مع المجموعة الضابطة $(18 \pm 0.612 \mu\text{M/L})$ في مصل النساء اللاتي يستعملن حبوب منع الحمل $(11.2 \pm 0.474 \mu\text{M/L})$ ، بينما لم تتأثر مستوى النحاس في مصل النساء اللاتي يستعملن اللولب 0.562 $(12.781 \pm \mu\text{M/L})$ بالمقارنة مع المجموعة الضابطة $(11.2 \pm 0.474 \mu\text{M/L})$ ، في حين انخفض مستوى فيتامين C في مصل كلا المجموعتين اللاتي يستعملن حبوب منع الحمل واللولب وبشكل معنوي $(P < 0.05)$ $(6 \pm 0.031 \mu\text{M/L})$ ، (5.57 ± 0.02) على التوالي بالمقارنة مع المجموعة الضابطة (10.73 ± 0.09) $\mu\text{M/L}$.

Introduction

Under normal condition, antioxidant mechanisms scavenge reactive oxygen species (ROS) and protect the organism from the damaging effects of oxidative stress, e.g. the one occurs with the administration of certain drugs (1). A biological antioxidant is a compound that protects a biological aerobic system against the potentially harmful effects of processes or reactions that can cause excess oxidation (2). In the living system and aerobic organism a complex of antioxidant mechanisms have been evolved to protect against uncontrolled free radical damage (3). These antioxidants act together in consent to form an integrated antioxidant system (4). Contraceptive agents are consumed by millions of women throughout the world. These agents, mostly sex steroids; influence the activity of many enzymes (1). The main copper transporter in blood is an α_2 -globulin protein called ceruloplasmin, exists in human plasma the acute-phase glycoprotein - ceruloplasmin (Cp) a 132kDa copper binding glycoprotein, it binds six or seven copper ions per molecule (5). Cp has been considered a type of plasma antioxidant due to its ability to react with and scavenge toxic oxygen species such as superoxide and hydrogen peroxide (6, 7, 8). Ceruloplasmin's antioxidant action may relate to its copper ion-binding ability. Copper-metal is a well-known prooxidant catalyst, and its

sequestration by ceruloplasmin, unrelated to its O_2^- scavenging activity and ferroxidase activity (9). Ceruloplasmin is a multifunctional enzyme, in addition Cp, as a growth factor, can be considered a regulatory function of the protein; it is mediated by the enzymatic ability of Cp to convert Fe (II) to Fe (III) thus preventing the Fenton reaction state (7, 10, 11, 12). Boyer and Schori (13) suggested that this enzymatic activity is required for the loading of Fe^{3+} into transferring and apoferritin. Vitamin C is a water-soluble antioxidant vitamin. It neutralizes free radicals in the plasma, cytoplasm and extracellular fluid (14, 15, 16). Cp oxidizes compound like ascorbic acid, epinephrine, melanonin, serotonin and other enzymes, and reduces levels of vitamin C (17, 18, 19). Recent studies indicate that certain side effects of administration of oral contraceptive have been attributed to chronic increase in serum ceruloplasmin which oxidizes compound like ascorbic acid, epinephrine, melatonin, serotonin and other amines (7, 14, 20). In view of these conflicting results, we aimed in the present study to investigate the plasma levels of Cp, copper, and vitamin C in women with administration of oral contraceptives, also to investigate the relationship between serum copper, and ceruloplasmin levels in these subjects.

Materials and Methods

Subjects: The study carried out on 120 women attending Family planning in Tikrit Teaching Hospital in Tikrit province. They were divided into 3 groups: group I: comprising forty women who were using oral contraception for at least 1 year, group 2: comprising forty women who were using copper T and group 3: consists of One hundred non- contraceptive users participated in the study as a control group. They were drawn from the same population and matched for age with the contraceptive-users group. Venous blood samples were taken from all subjects and were left to clot then centrifuged at 3000 rpm for 10 minutes; the blood serum samples were obtained and were preserved at -20°C temperature till the laboratory analysis was done by the colorimetric method.

Estimation of Cp⁽²¹⁾: At pH 5.4, CP catalyzes the oxidation of para phenylene diamine (PPD) to yield colored oxidation product. The formation rate of the colored oxidation product is proportional to the concentration of serum ceruloplasmin.

Determination of serum copper⁽²²⁾: Copper is an essential human nutrition and a component of many metalloenzymes. At pH 4.7 copper, which is bond to ceruloplasmin is released by reducing agent (3, 4-Dibromo-2-Pyridylazo)-N-Ethyl-N-(3-Sulphopropyl) alanine, to form a stable colored chelate. The intensity of the color is directly proportional to the amount of copper in the sample.

Determination of serum vitamin C by HPLC Technique⁽²¹⁾.

Statistical analysis: statistical comparison was performed by using t

test and X² statistics for nonparametric ones. P value of less than 0.05 was considered significant.

Results

Forty women in reproductive age group were using oral contraceptive (group I) for at least 1 year, 40 women were using copper T(group II) and 40 healthy women served as control(group III) were enrolled in the present study. The average age of group I was 33.08± 2.327 years. The results of study showed significantly high serum ceruloplasmin level in among group I in comparison with group III (, P<0.05), therefore the mean ± SE of serum Cp was (60±0.16 mg/dl vs. 36.5±0.96 mg/dl respectively).while the serum (Cp) were not significantly different between group II and group III (37.29±0.99 mg/dl vs. 36.5±0.96 mg/dl P<0.05)(Table1). Women in group I had also significantly higher serum copper levels (P<0.01) as compared to group III. The mean of serum copper level in group I and group III was 18±0.612 µM/L vs, 11.2±0.474 µM/L respectively. While the serum copper were not significantly different between women in group II and group III(12.781±0.562 µM/L, vs, 11.2±0.474 µM/L, (P<0.01) (Table2) . On the other hand the serum vitamin C values in group I and group II (5.57±0.02 , 6±0.031 µM/L respectively) were significantly lower than group III(10.73± 0.09 µM/L, P<0.05) (Table3).

Table (1):- Effect of different contraceptives methods of contraception on serum ceruloplasmin.

	No. of cases	Mean & SD ceruloplasmin
Group I	40	60±0.16 mg/dl
Group II	40	37.29±0.99 mg/dl
Group III	40	36.5±0.96 mg/dl
P<0.05		

Table (2):- Effect of different contraceptives methods of contraception on serum copper.

	No. of cases	Mean & SD copper
Group I	40	18±0.612 µM/L
Group II	40	12.781±0.562 µM/L
Group III	40	11.2±0.474 µM/L
P<0.01		

Table (3):- Effect of different contraceptives methods of contraception on serum vit.C

	No. of cases	Mean & SD vit.C
Group I	40	5.57±0.02 µM/L
Group II	40	6±0.031 µM/L
Group III	40	10.73± 0.09 µM/L
P<0.05		

Discussion

The elevated value of ceruloplasmin after administration of oral contraceptive in present study is in accordance with other workers^(20, 23, 24). Certain side effects of oral contraceptive have been attributed to chronic increase in serum ceruloplasmin⁽²⁵⁾. The estrogen component is mainly responsible for the increased level of serum ceruloplasmin while progesterone cause a less drastic rise⁽²⁶⁾. Considering the operon concept of Monod and Jacob estrogen act as inducer for synthesis of ceruloplasmin RNA templates causing subsequent

increase in synthesis of the protein⁽²⁵⁾. Ceruloplasmin act as cis antioxidant through either prevention of decompartmentised iron acting as free radical catalyst or by directly inactivating free radicals escaped from neutrophil to extracellular fluid which lacks catalase or superoxide dismutase⁽²⁷⁾. Recent evidence suggests that Cp exhibits potent prooxidant activity and causes oxidative modification of important biomolecules like low density lipoprotein. This newly described prooxidant, activity of Cp may help to explain epidemiological studies indicating that Cp is an independent risk factor for cardiovascular disease^(28, 29). In present

study, mean of serum copper among women were using contraceptive pills is significantly higher ($P < 0.01$) than the mean of control. High level of serum copper that is associated with low concentration of Cp and iron in the liver and causes increased free radical production⁽³⁰⁾. Other factors, such as sex, hormonal state, diet and geographical differences are known to affect serum copper levels. Socio-cultural and genetic factors may also affect serum copper and ceruloplasmin levels indirectly. For example, in communities where the elderly are taken care of at home rather than in institutions, better diet in the former may affect the serum levels of copper and ceruloplasmin⁽³¹⁾. Hence women taking oral contraceptives pills may be considered a high risk group. Secondly Cp also oxidizes compound like ascorbic acid, epinephrine, melatonin, serotonin and other amines. Under physiological conditions this oxidation is minimized by common metabolic citrate. Any condition leading to rise in serum Cp can lead to increased oxidation of the above mentioned substrates^(32, 33). Reduced levels of vitamin C have been detected in the serum of women taking oral contraceptives with a mean reduction of 30-40%^(17, 20, 34). The use of oral contraceptives has been consistently associated with decreased levels of vitamin C as a result of their interference with the metabolism of ascorbic acid^(35, 36). Several studies indicate that women receiving oral contraceptives are in induced hypovitaminotic C condition due to raised serum ceruloplasmin^(17, 20, 34). Women taking oral contraceptives could reduce their adverse effects upon ascorbate levels through supplementation with vitamin C. While suggested supplemental dose for ascorbate is usually 500-1000 mg per day, higher levels in the range of 1000-

2000 mg per day may be indicated in instances such as the use of oral contraceptives^(35, 37). The results showed a correlation between serum copper and ceruloplasmin level among women using contraceptive pills, also serum I vitamin C levels decreased when serum ceruloplasmin level increase. Further studies are required, with a larger sample size taking into account the effect of age, also to estimate the level of zinc contents in using contraceptive pills.

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