Evaluation of serum ceruloplasmin, copper, iron and Vit.C levels in Women using oral contraceptive pills in Tikrit city

* Intidhar R. Sarhat, ** Ayhan R. Mahmood, ***Safaldin, A. Abed
* Department of Biochemistry, College of Dentistry, University of Tikrit, Tikrit, Iraq
** Department of Biochemistry, College of Dentistry, University of Tikrit, Tikrit, Iraq
***Dijlah Rehabilitation Hospital, Tikrit, Iraq

Received 19/2/2008 accepted 22/4/2008

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, α2-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 54 women who were using oral contraceptives for at least 1 year. The mean age of women was (32.08 years vs. 27.04 years of control). The serum level of ceruloplasmin (Cp) as antioxidant protein was significantly high in comparison to control group (mean 40.5 mg/dl vs. 33.28 mg/dl, P<0.001), also mean of serum copper in women who were using contraceptive (17.03 µM/L) is significantly higher (P<0.01) than the mean of control (12.991 µM/L). On the other hand the serum vitamin C values in women using contraceptives were significantly lower than control group (P<0.05), the mean of vitamin C value was 3.452 µM/L vs. 6.534 µM/L of control. The mean value of serum iron in women were using contraception (8.642 µM/L) which is less significantly (P<0.01) than the mean value of the control group (17.603 µM/L).

تقدير مستوى سيريلوبلاسمين والنحاس والحديث وفيتامين C في مصل النساء اللاتي يتعاطين مواد الحمل في مدينة تكريت

سيف الدين أحمد عبد

المستخلص

يبلغ عدد النساء اللاتي يتعاطين مواد الحمل من 60-70 مليون واجريت دراسات عديدة عن مدى الخطورة الناجمة عن استعمال هذه الأدوية. كما وجدت تقارير عن تأثير هذه الأدوية في خفض فعالية بعض الأنزيمات كسيريلوبلاسمين (وهو عبارة عن ألفا كليوبلامين وهو مركب رئيسي في نقل النحاس) وفيتامين C. أجريت الدراسة الحالية في وحدة تنظيف العائلة في مستشفى تكريت العام، إذ شملت الدراسة 54 امرأة تعاطي مواد الحمل إذ بلغ معدل أعمارهن (32.08) سنة و4.54 أقرأة في معدل الضربة بلغ معدل أعمارهن 27.04 سنة. تم جمع عينات الدم من المجموعة لغرض تقدير فعالية سيريلوبلاسمين والنحاس والحديث وفيتامين C في مصل النساء اللاتي يتعاطين مواد الحمل من مجموعة Comparisons between Mean Levels of Serum Ceruloplasmin, Copper, Iron and Vitamin C in Women Using Oral Contraceptive Pills in Tikrit City (P≤0.001) (17.03 µM/L) which is significantly higher (P<0.01) than the mean of control (12.991 µM/L). In the group of under the age of 35 years, the mean level of copper was 15.50 µM/L vs. 13.27 µM/L of control. On the other hand the serum vitamin C values in women using contraceptives were significantly lower than control group (P<0.05), the mean of vitamin C value was 3.452 µM/L vs. 6.534 µM/L of control. The mean value of serum iron in women were using contraception (8.642 µM/L) which is less significantly (P<0.01) than the mean value of the control group (17.603 µM/L).
Introduction
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 α2-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 (2). 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 (3-5).

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 O2 scavenging activity and ferrooxidase activity (6). Cp 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 (4,7,8,9) Boyer and Schori (10) suggested that this enzymatic activity is required for the loading of Fe3+ into transferring and apoferritin. Vitamin C is a water-soluble antioxidant vitamin. It neutralizes free radicals in the plasma, cytoplasm and extracellular fluid (11,12,13). Cp oxidizes compound like ascorbic acid, epinephrine, melatonin, serotonin and other enzymes, and reduces levels of vitamin C (14,15,16).

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 (14,11,17). In view of these conflicting results, we aimed in the present study to investigate the plasma levels of Cp, copper, iron, 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 108 women attending Family planning in Tikrit Teaching Hospital in Tikrit province. They were divided into 2 groups: group I comprising fifty four women who were in the reproductive age ranged between 20-39 years, all of them were using oral contraception for at least 1 year, and group II control group consists of fifty four women with age varies between 19-37 years. 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. 

Determination of serum copper (19): 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 (19): 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 iron (20): Ferric iron is dissociated from its carrier protein, transferring, in an acid medium and simultaneously reduced to the ferrous form. The ferrous iron is then complexes with the chromogen, a sensitive iron indicator, to reduce a blue chromophore, which absorbs maximally at 595 nm. Determination of
serum vitamin C by HPLC Technique (18). Statistical analysis: statistical comparison was performed by using t test and X^2 statistics for nonparametric ones. P value of less than 0.05 was considered significant.

Results
Fifty four women in reproductive age group were using oral contraceptive for at least 1 year, and 54 healthy women served as control were enrolled in the present study. The average age of group I was 32.08± 2.327 years whereas the average age of group II was 27.04± 1.196 years. The results of study showed significantly high serum ceruloplasmin level in among group I in comparison with group II (P<0.001), therefore the mean ± SE of serum Cp was 40.5 ±3.734 mg/dl vs. 33.28 ±2.52 mg/dl of control group (figure 1).

Women in group I had also significantly higher serum copper levels (P<0.01) as compared to group II. The mean of serum copper level in group I and group II was 17.03 ±0.522 μM/L, 12.991 ±0.582 μM/L respectively (figure 2).
The study also demonstrated significant difference in serum iron levels between both groups (P<0.01). The serum iron levels in group I were less significantly than of the group II (8.642 ±0.320 μM/L versus 17.603 ±0.613 μM/L) as showed in figure (3).

The results showed significantly lower level of (P<0.05) serum Vit.C activity in group I than group II. The mean of serum vitamin C level in contraceptive women was 3.452 ±0.165 μM/L versus 6.534 ±0.242 μM/L in control group, (figure 4).

**Discussion**

The elevated value of ceruloplasmin after administration of oral contraceptive in present study is in accordance with other workers (11, 21). Certain side effects of oral
contraceptive have been attributed to chronic increase in serum ceruloplasmin (23). The estrogen component is mainly responsible for the increased level of serum ceruloplasmin while progesterone cause a less drastic rise (24). 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 (23). Ceruloplasmin act as cis antioxidant through either prevention of decompartmentalized iron acting as free radical catalyst or by directly inactivating free radicals escaped from neutrophil to extracellular fluid which lacks catalase or superoxide dismutase (25). 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 (26, 27). 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 (28). Other factors, such as sex, hormonal status, 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 (29). Decreases levels of serum iron among group I in present study is due to the presence of high level of ceruloplasmin, which convert the toxic “Ferrous” iron to its non-toxic form, “Ferric” (8, 30). Iron has the capacity to accept and donate electron readily, this capability makes it physiologically essential, as useful component of cytochromes and oxygen-binding molecules. However, Iron is also biochemically dangerous, it can damage tissue by catalyzing the conversion of H2O2 to free-radical ions that attack cellular membranes lipids, proteins and DNA (31, 32). 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. Reduced levels of vitamin C have been detected in the serum of women taking oral contraceptives with a mean reduction of 30-40% (14, 17, 33). 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 (34, 35). Several studies indicate that women receiving oral contraceptives are in induced hypovitaminotic C condition due to raised serum ceruloplasmin (14, 17, 33). 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 (34, 36).

**Conclusion**

The results showed a correlation between serum copper and ceruloplasmin level among women using contraceptive pills, also serum iron and vitamin C levels.
decreased when serum ceruloplasmin level increase. Recommendation: 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.

References
Prevalence of hypocalcemia among thalassemic patients registered in ibn al-balady hospital

*Dr. Ali Hasan Dhary Al- Jumaili,**Dr. Shaimaa Khider,**Dr. Waseem Ali Hasan,
*Central Teaching Hospital Children, Iraq- Baghdad
**Ibn Al-balady Hospital (Thalassemic Centre) Iraq- Baghdad,
***College of Pharmacy, University of Tikrit, Tikrit, Iraq.

Received 9/11/2008 accepted 30/12/2008

Abstract
The objective of this study is to through light on the prevalence of hypocalcaemia in thalassemic patients registered in Ibn Al-balady Hospital (Thalassemic Centre) Baghdad-Iraq four hundred patients selected with thalassemia major were included in the study, randomly selected throughout October, 2001. They are subjected to serum study for calcium phosphorus and alkaline phosphatase. Twenty patients x-rayed for bones only no facilities for studying serum ferritin, parathyroid hormone and densometry for bones. It was found that hypocalcaemia is prevalent in eighty seven patients out of 400 and more prevalent among age group 10 years and above.

Antashar Nqassl al-kalssyoom biin al-marosyim almu'sabibin bimrass al-thalaszyima في مستشفى ابن البلدي
علي حسن
شيما خضر
وسام علي

المستخلص
إن الهدف من هذه الدراسة هو دراسة انتشار نقص الكالسيوم في مرضى الثالاسيميا حيث سجلنا هذه الدراسة في مستشفى ابن البلدي (مركز الثالاسيميا) - بغداد- العراق. لغرض تحقيق نتائج الدراسة اختيار أربع مائة مريض مصابين في سن الثالاسيميا الرئيسية، تم اختيارهم بشكل عشوائي طوال الشهر أكتوبر وتشرين الأول لعام 2001. هذه العينات تم إخضاعها إلى دراسة عناصر الفسفور، الكالسيوم ونسبةatem اللفاكتور في صلصة الدم. حيث وجد أن انخفاض مستوي الكالسيوم سائد في سبعين وثمانين مريض من المجموعة المثلثة والذي هو ارتعاش مريض وأكثر شيوعا بين المجموعات العمرية ذات العشر سنوات وما فوق.