

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

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### Abstract

Oral contraceptive steroids are used by an estimated 60 to 70 million women world-wide. 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,  $\alpha_2$ -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 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 were using contraceptive (17.03  $\mu\text{M/L}$ ) is significantly higher ( $P < 0.01$ ) than the mean of control (12.991  $\mu\text{M/L}$ ). 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  $\mu\text{M/L}$  vs. 6.534  $\mu\text{M/L}$  of control. The mean value of serum iron in women were using contraception (8.642  $\mu\text{M/L}$ ) which is less significantly ( $P < 0.01$ ) than the mean value of the control group (17.603  $\mu\text{M/L}$ ).

تقدير مستوى سيريلوبلازمين والنحاس والحديد وفيتامين C في مصل النساء اللاتي يتعاطين موانع الحمل 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 كليبولين وهو مركب رئيسي في نقل النحاس)، وفيتامين C. أجريت الدراسة الحالية في وحدة تنظيم العائلة في مستشفى تكريت العام، إذ شملت الدراسة 54 امرأة يتعاطين موانع الحمل إذ بلغ معدل أعمارهن (32.08) سنة و54 امرأة في مجموعة الضابطة بلغ معدل أعمارهن 27.04 سنة. تم جمع عينات الدم من المجموعتين لغرض تقدير فعالية سيريلوبلازمين والنحاس والحديد وفيتامين C في المصل. أظهرت نتائج الدراسة الحالية ارتفاع معدل مستوى السيريلوبلازمين (40.5 mg/dl) وبشكل معنوي ( $P < 0.001$ ) في مصل المجموعة اللاتي يستعملن موانع الحمل بالمقارنة مع المجموعة الضابطة (33.28 mg/dl) كما ارتفع مستوى النحاس في مصل النساء اللاتي يتعاطين موانع الحمل (17.03  $\mu\text{M/L}$ ) وبشكل معنوي ( $P < 0.01$ ) بالمقارنة مع المجموعة الضابطة (12.991  $\mu\text{M/L}$ ). في حين انخفض مستوى الحديد في مجموعة المستخدمة موانع الحمل وبشكل معنوي ( $P < 0.01$ ) إذ بلغ معدل الحديد في المصل (8.642  $\mu\text{M/L}$ ) بينما بلغ في المجموعة الضابطة (17.603  $\mu\text{M/L}$ ). كما وانخفض مستوى فيتامين C في مصل النساء اللاتي يتعاطين موانع الحمل وبشكل معنوي ( $P < 0.05$ ) حيث بلغ معدل فيتامين C في المصل (3.452  $\mu\text{M/L}$ ) أقل من المجموعة الضابطة الذي بلغ معدل فيتامين C في مصل (6.534  $\mu\text{M/L}$ ).



## Introduction

Contraceptive agents are consumed by millions of women throughout the world. These agents, mostly sex steroids; influence the activity of many enzymes<sup>(1)</sup>. The main copper transporter in blood is an  $\alpha$ 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<sup>(2)</sup>. 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<sup>(3, 4, 5)</sup>. 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<sup>(6)</sup>. 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<sup>(4, 7, 8, 9)</sup>. Boyer and Schori<sup>(10)</sup> 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<sup>(11,12,13)</sup>. Cp oxidizes compound like ascorbic acid, epinephrine, melanonin, serotonin and other enzymes, and reduces levels of vitamin C<sup>(14, 15, 16)</sup>. 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<sup>(4, 11, 17)</sup>. 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

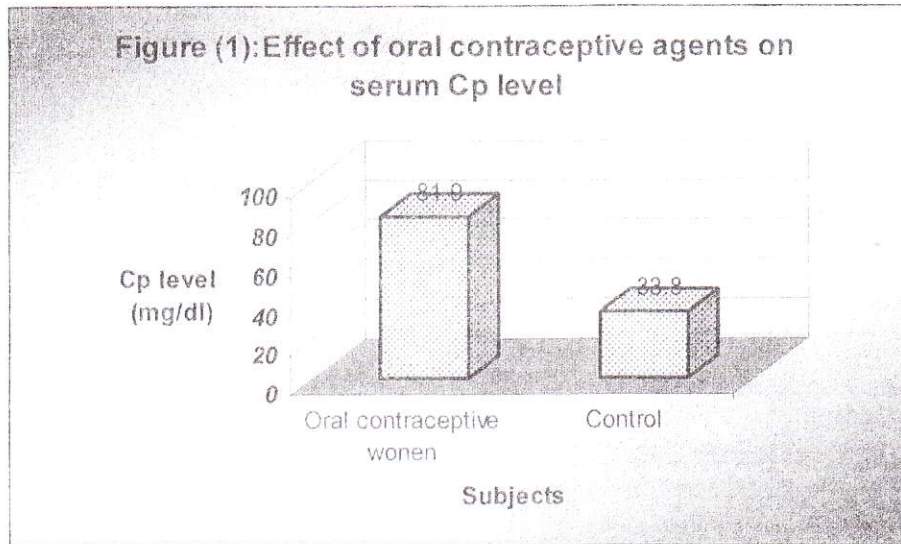
**Subjects:** The study carried out on 108 women attending Family planning in Tikrit Teaching Hospital in Tikrit province. They were divided into 2 groups: group I comprising fifty four women who were in the reproductive age ranged between 20-39 years, all of them were using oral contraception for at 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  $-20^\circ C$  temperature till the laboratory analysis was done by the colorimetric method. **stimation of Cp<sup>(18)</sup>:** 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<sup>(19)</sup>:** 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<sup>(20)</sup>:** 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<sup>2</sup> statistics for nonparametric ones. P value of less than 0.05 was considered significant.

### Results

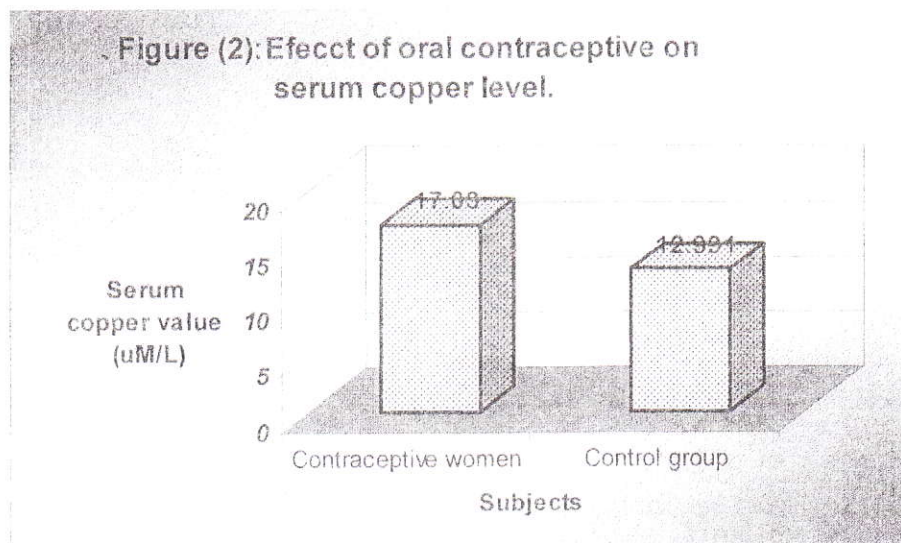
Fifty four women in reproductive age group were using oral contraceptive for at least 1 year, and 54 healthy women

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

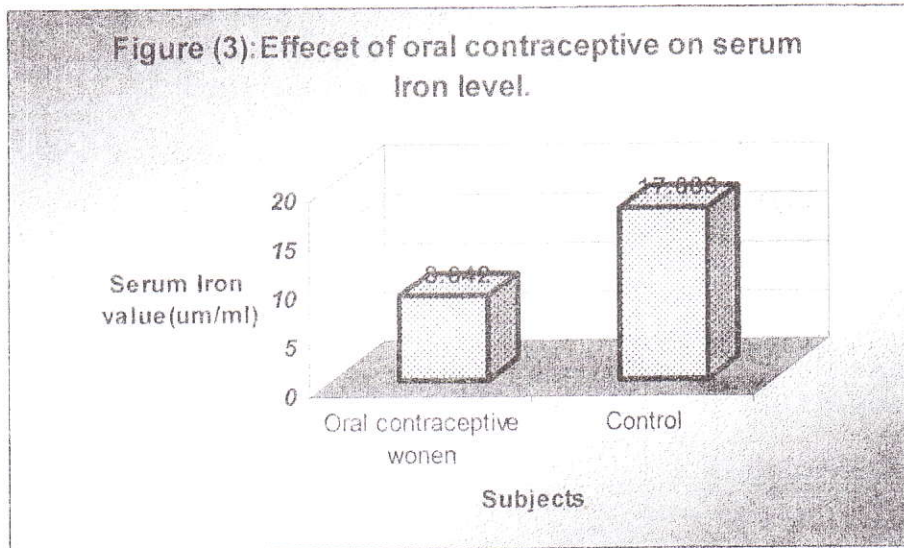
group I and group II was 17.03 ±0.522 μM/L, 12.991 ±0.582 μM/L respectively (figure 2).





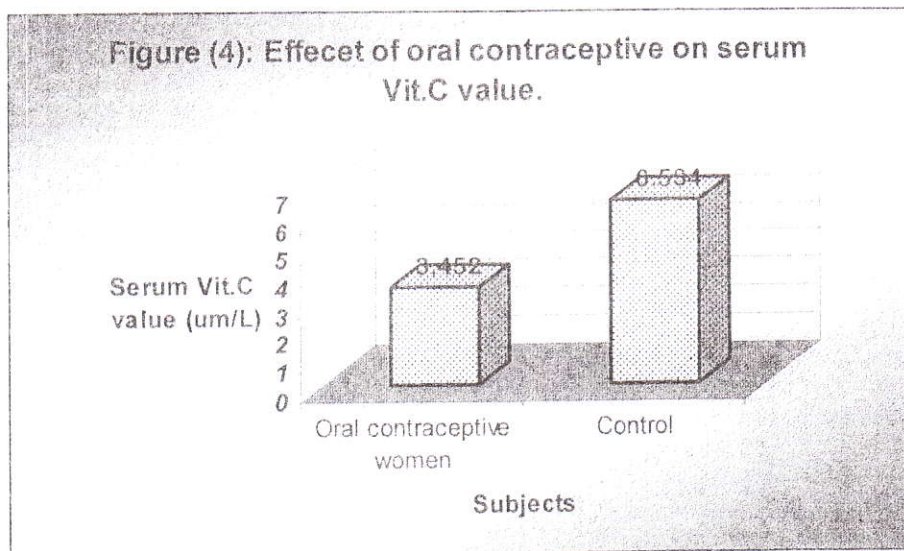
The study also demonstrated significant difference in serum iron levels between both groups ( $P \leq 0.01$ ). The serum iron levels in group I were

less significantly than of the group II ( $8.642 \pm 0.320 \mu\text{M/L}$  versus  $17.603 \pm 0.613 \mu\text{M/L}$ ) as showed in figure (3).



The results showed significantly lower level of ( $P \leq 0.05$ ) serum Vit.C activity in group I than group II. The mean of serum vitamin C level in contraceptive

women was  $3.452 \pm 0.165 \mu\text{M/L}$  versus  $6.534 \pm 0.242 \mu\text{M/L}$  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 (17, 21, 22). Certain side effects of oral

contraceptive have been attributed to chronic increase in serum ceruloplasmin<sup>(23)</sup>. The estrogen component is mainly responsible for the increased level of serum ceruloplasmin while progesterone cause a less drastic rise<sup>(24)</sup>. 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<sup>(23)</sup>. 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<sup>(25)</sup>. 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<sup>(26, 27)</sup>. 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<sup>(28)</sup>. 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<sup>(29)</sup>. 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"<sup>(8, 30)</sup>. 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  $H_2O_2$  to free-radical ions that attack cellular membranes lipids, proteins and DNA<sup>(31, 32)</sup>. 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%<sup>(14, 17, 33)</sup>. 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<sup>(34, 35)</sup>. Several studies indicate that women receiving oral contraceptives are in induced hypovitaminotic C condition due to raised serum ceruloplasmin<sup>(14, 17, 33)</sup>. 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<sup>(34, 36)</sup>.

### 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.

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

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### 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

انتشار نقص الكالسيوم بين المرضى المصابين بمرض الثلاسيميا في مستشفى ابن البلدي

علي حسن      شيماء خضير      وسيم علي

### المستخلص

إن الهدف من هذه الدراسة هو دراسة إنتشار نقص الكالسيوم في مرضى الثلاسيميا حيث سجلت هذه الدراسة مستشفى ابن البلدي (مركز الثلاسيميا) - بغداد- العراق. تضمنت هذه الدراسة اختيار أربعمائة مريض يعانون من الثلاسيميا الرئيسية ، تم اختيارها بشكل عشوائي طوال الأشهر أكتوبر و تشرين الأول لعام ٢٠٠١ . هذه العينة العشوائية تم اخضاعها إلى دراسة عناصر الفسفور ، الكالسيوم وانزيم الفوسفاتيز القلوي في مصل الدم. حيث وجد ان انخفاض مستوى الكالسيوم سائد في سبعة وثمانون مريض من المجموع الكلي والذي هو اربعمائة مريض وأكثر سيادة بين المجاميع العمرية ذات العشر سنوات فما فوق.