STUDY OF SOME IMMUNOLOGICAL PARAMETERS OF IRAQI WOMEN TAKING COMBINED ORAL CONTRACEPTIVES

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Abstract
Combined oral contraceptive (COC) caused many immunological abnormalities; our study involved 25 woman taking COC one sheet/month and 20 women not use COC (control). The women were examined for acute phase proteins (caeruloplasmin, human alpha antitrypsin (αAT) and C-reactive protein), also for antinuclear antibodies (ANA) and body mass Index (BMI).

Our results showed significant increase in the level of caeruloplasmin, αAT and Crp in COC users which are 486.8mg/dL, 1889.6mg/L and 7.79mg/L respectively compared with the control (non COC users) 355.9mg/dL, 1591.8mg/L and 3.9mg/L. Also results showed high significant increase in ANA level of COC users with a mean of 54.88 u/ml compared with control 36u/ml. BMI showed increase but not significant, in this study it appeared the relationships between ANA, Crp and BMI, and between Caeruloplasmin, ANA, and also BMI, Crp with pearson correlation (r) respectively 0.395, 0.373, 0.349, P > 0.01. The increase of acute phase proteins were associated with the incidence of cardiovascular diseases, and the increase in the level of ANA was related to be more susceptibility to autoimmune diseases.
Introduction

Oral contraceptive pills are synthetic steroids (hormones). They are reversible birth control in the word, are resemble to that in woman but the natural hormones are rapidly metabolized in liver and become useless for contraception[1,2]. There are two types of oral contraceptive pills (OCP) first type called combined oral contraceptive (COC) contain two hormones that are estrogen and progestin, the second type called progesting oral pills (POP) contain only progestin[3].

Combined oral contraceptive (COC) is the most commonly used in Iraq. Prolonged use of COC like (nordette) cause many biological and humoral immunological abnormalities for that reason we focus on several humoral component as antinuclear antibodies (ANA) and acute phase proteins (immunological assayed serum proteins) like C-reactive protein, careruloplasmin and human alpha 1 antitrypsin (α1AT).

COC: the form of birth control suppresses ovulation (the monthly release of an egg from the ovaries) by the combined action of the hormones estrogen and progestin. If a woman remembers to take the pill every day as directed; she has an extremly low chance of becoming pregnant in a year [4]. But the effectiveness may be reduced if the women is taking some medications, such as certain antibiotics [5].

Besides, preventing pregnancy the pill can make periods more regular, it also has protective effect against pelvic inflammatory diseases, an infections of the fallopian tubes or uterus that is major cause of infertility in women and against ovarian and endometrial cancer [6].

Also it was used to treat some cases like acne, hirsutism, and polycystic ovarian syndrome [7]. Disadvantage Birth control pills are considered safe for most women but they carry some risk for women who smoke especially those over 35 years and women with certain medical conditions such as history of blood clots or breast or endometrial cancer, hypertension, diabetes, epilepsy, breast feeding through the first six months because (COC) inhibited production of milk [2,8] may be advised against taking the pill. The side effects include nausea, headache, breast tendness, weight gain, irregular bleeding, depression, alternations in triglycerides level, cholesterol [9].

This side effect often subside after a few months use of the pills [2].

Generally COC more efficient than POP because it has less disadvantage, in the same time POP is ideal for women who cannot take (COC) like breast feeding, old women, presence of cardiovascular risk factor, diabetes[3].

Combined oral contraceptive and Immune system COC appear to influence the immune system for example suffer of higher incidence of autoimmune diseases such as induce systemic lupus erythymatosus[10,11], and depresses the immune system was exposed by testing immune function like immunoglobulin production, cell mediated response by phytoheamagglutinin (PHA)[12].

Some studies focus on previous maternal use of COC (before pregnancy) lead to birth children more susceptible to some allergic diseases like asthma, allergic rhinitis, atopic eczema more than mothers who don’t take COC [13].

Caeruloplasmin is a 135KDα - Z serum glycoprotein synthesised in the liver it has a major role in metabolism of copper (to which it can bind reversibly) and 95% of copper in plasma is carried by this protein caeruloplasmin also acts as a ferroxidase and superoxide dismutase and thereby protects polysaturated fatty acid red blood cell membranes from active oxygen radicals[14].

Human alpha 1 antitrypsin (α1AT), α1AT is a 135KD serum glycoprotein that is synthesised by hepatocytes and to a lesser extent by mononuclear phagocytes. It is a serine protease inhibitor acting principally on neutrophil elastase, thereby protecting the lung from degradation by this enzyme [15,16].

Material and Methods

Two Groups of Woman were investigated, Group A. Women taking COC (germany nordette): A total of (25) samples were collected from family planning clinic of Al-Salame healthy center in Baghdad. The rang age of volunteers is (23-45) year and they took one sheet/month which contains 21 pills each one contain levonorestrol 0.25mg and ethiny1 estradiol 0.05mg and 7 pills of ferrous fumarate.

Volunteers are in reproduction age and have no chronic diseases. Group B. Control: Apparently healthy control: A total number of (20) samples that do not take pill and are not pregnant, the age of this group
approached to test group age (in reproduction age).

Methods
1. Acute phase proteins
   - **Caeruloplasmin and alpha1 antitrypsin (α1AT):**
     Caeruloplasmin and α1AT were examined by radial immune diffusion assay. Principle of the assay: the method involved antigen diffusing radially from acylindrical well through and agarose gel containing an appropriate monoclonal antibody. Antigen-antibody complexes are formed when under the right condition will form a precipitin ring. These tests were performed as described in the leaflet of the kit (Binding site uk).

   **C-reactive protein (CRP)**
   Principle: the latex reagent is a suspension of polystyrene latex particles of uniform size coated with the IgG fraction of an anti-human CRP, specific serum. Latex particles allow visual observation of Ag-Ab reaction. These tests were performed as described in the leaflet of kit (labkit, Barcelona). The serum contains approximately more than (6mg/L) of CRP when a clear agglutination when a clear agglutination become evident.

   **Semiquantitative test for CRP**
   The test was performed in the same way of qualitative test but using previous dilution of the serum sample in normal saline. (Table 1).

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>1/2</th>
<th>1/4</th>
<th>1/8</th>
<th>1/16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample serum</td>
<td>100μL</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Saline</td>
<td>100μL</td>
<td>100μL</td>
<td>100μL</td>
<td>100μL</td>
</tr>
<tr>
<td>Volume of sample</td>
<td>50μL</td>
<td>50μL</td>
<td>50μL</td>
<td>50μL</td>
</tr>
</tbody>
</table>

Concentration will be the reciprocal of positive result dilution.

Table (1): Semiquantitative test for CRP.

<table>
<thead>
<tr>
<th>6 x n. of dilution</th>
<th>6 x 2</th>
<th>6 x 4</th>
<th>6 x 8</th>
<th>6 x 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/L</td>
<td>12</td>
<td>24</td>
<td>48</td>
<td>96</td>
</tr>
</tbody>
</table>

**Antinuclear antibodies (ANA)**
These constitute adverse group of antibodies specificities against intracellular antigens. These antibodies (ANA) were detected by ELISA assay.

**Principle of test**
The test is based on the covalent immobilization of Hela cell nuclei to the solid phase of microtiter strips and subsequent binding of ANA from patient serum. The bound antibodies are detected with a peroxidase – labeled secondary antibody that is directed against human IgG, IgM and IgA. After addition of substrate solution a color appears with intensity proportional to the concentration and/or the avidity of the detected antibodies. Following the addition of stop solution, the colour switches from blue to yellow. This test was performed as described in the leaflet of the kit (Imtec Human).

Normal values:
- < 40 u/ml Negative
- 40-55 u/ml equivocal
- > 55 u/ml positive

**Body Mass Index (BMI)**
BMI was defined as weight in kilogram/height in square meters, this index should be documented for women in using COC.

Results and Discussion
1. Acute phase proteins
   A. Caeruloplasmin: Data show significant increase in caeruloplasmin level in (COC) users (mean 486.8mg/dL) compared to control (mean 355.9mg/dL) P < 0.001 this result is similar to those of [17, 18], Figure. 1, table 2.

![Figure 1: Distribution of caeruloplasmin level among COC users.](image)

This increase may be associated with the natural function of caeruloplasmin it is maintainence
cellular iron level, in other words caeruloplasmin is important for normal release of cellular iron, the irregular bleeding in women taking COC need to this action [19]. In the same time this protein is important for copper metabolize thereby for hemaoglobin formation and iron transport, that examined in last study appeared increase in level of copper in COC users compared with non users[18], copper is the rate-limiting element in synthesis caeruloplasmin [20]. The prolonged taking of COC is associated with severe increase of caeruloplasmin that rise risk of cardiovascular disease [21], also the administration of COC assotiated with epidermoid diseases [3]. This may be associated with increasing of this protein in women that had cases called hypercoppercamia[22].

Human alpha 1 antitrypsin (α1AT)

The results showed significant increase in this glycoprotein (mean 1889.6 mg/L) in COC users compared to control (1591.8mg/L) P < 0.001 Figure. 2. These results are in agreement with [23, 24, 25].

This increase in α1AT may be capable of this protein to increase it's concentration in serum in response to physiologic stresses such as sex hormones (estrogen hormone), pregnancy and diabetes [23, 26]. This increase limits proteolysis by protease. The relationship between this protein and COC clear in studies examined α1AT deficiency, in these studies willing to use contraceptives throughout study [27]. If human have an imbalance between the neutrophil elastase in the lung (protease) and the antielastase (anti α1AT) that are responsible for defecte in lung which can lead to clinical condition called emphysema [15, 16, 28]. In other word this increase of antitrypsin due to limit the protolysis of protease to inhibit it and formation of an enzymatically action complex that involves the active site of protease (17, 29).

C-reactive protein (CRP)

This study showed significant increase in CRP level mean (7.79 mg/L) compared with control (3.9 mg/L) P < 0.01 (Figure. 3) the percentage of positive cases is 28% compared with 0% of control these results similar to (21, 30, 31), this increase may due to ability of estrogen to induced some changes like haemostasis, venous throumbosis and inflammation risk factors (one of them crp)[32].

![Figure 2: Distribution of α-1anitrypsin level among studying groups.](image2)

![Figure 3: Distribution of CRP level among studying groups.](image3)

2. ANA

The current study showed that the association between the use of COC and the induction of ANA the mean of titer is (54.884 u/ml compared with control (mean 36.44 u/ml) Figure. (4). P < 0.001, the percentage of positive cases is 44% compared with control 0% this results similar to[33] the synthetic estrogenor progestagene found in COC might induce similar serologic change and suffer a higher incidence of autoimmune diseases[34, 11].

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Caeruloplasmin</th>
<th>αAT</th>
<th>Crp</th>
<th>ANA</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test (COC users)</td>
<td>25</td>
<td>486.8 ± 144.5</td>
<td>1889.6</td>
<td>7.76</td>
<td>54.88</td>
<td>46.0 ± 5.71</td>
</tr>
<tr>
<td>Control (non COC users)</td>
<td>20</td>
<td>355.9 ± 97.02</td>
<td>1591.8</td>
<td>3.9</td>
<td>36.3</td>
<td>42.755</td>
</tr>
</tbody>
</table>

Table 2: Mean and std. deviation of studied groups.
Body Mass Index (BMI)
This study showed increase in this index but the results were not significant (test mean 46 kg/mm$^2$) compared with control mean 42.7 kg/mm$^2$) that established in previous studies like [35] (Figure 5).

Relationships between parameters of examined groups
In our study the observed relationships between ANA and CRP with Pearson correlation (r) 0.395 P < 0.001 (Figure 6), and relationship between ANA and caeruloplasmin with person correlation (r) 0.373 P< 0.01 (Figure. 7), also found correlation between BMI and Crp with pearson correlation (r) 0.349 P < 0.01 (Figure. 8),there results are similar to previous study[36].

References
4. Fraser, T. S. 2000. Forty years of combined oral contraception: The evolution of a


28. Taus; G.; Bouma, BN.; Bulker, HR and Rosing J. 2003. Changes of haemostatie