The Effect of Combined Oral Contraceptive Pills and Copper Bearing Intrauterine Contraceptive Devices on The Oxidative Stress, Lipid Profile and Some Trace Elements in Women Sera in Hilla City

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Abstract

The study was conducted on sixty three healthy women used combined oral contraceptive pills (COCPs) and copper bearing intrauterine contraceptive devices (IUDs). Thirty women were used Microgynon® (low dose COCPs, each pill containing levonorgestrel 0.15 mg and ethinyl estradiol 0.03 mg) and thirty three women used copper T 380A IUDs. Thirty apparently healthy women were taken as control group. Blood samples obtained from women used contraceptives and control group from Babylon maternity and pediatric hospital in Hilla city. The sera obtained from the blood were used to determine the effect of COCPs and IUDs on of malondialdehyde (MDA), total cholesterol, high density lipoprotein (HDL), triglycerides (TGs), very low density lipoprotein (VLDL), low density lipoprotein (LDL) and some trace elements (copper, zinc and iron) concentration.

The results of the present study showed (significant increase in MDA, copper, iron, total cholesterol, TGs, VLDL and LDL concentration, significant decrease in HDL and non significant decrease in zinc concentration in sera of women who used COCPs when compared to both women who used IUDs and the control group. Also this study showed non significant difference in MDA, copper, zinc, total cholesterol, HDL, TGs, VLDL and LDL concentration and significant decrease in iron concentration in sera of women who used IUDs when compared to those of the control group.
Introduction

Contraception means the ability to control fertility by reliable artificial methods has transformed both social and epidemiological aspects of human reproduction [1]. Men and women have used contraception, in one form or another, for thousands of years. There is no one method that will suit every one and individual will use different types of contraceptives at different stage of lives [2].

Combined oral contraceptive pills (COCPs) are medicines taken by mouth to prevent pregnancy by prevent ovulation by suppressing the release of gonadotropins (follicle-stimulating hormone and greatly luteinizing hormone) [3, 4]. COCPs are highly effective, reversible and popular. They also have noncontraceptive uses, including control of menstrual abnormalities, as these agents decrease blood loss, decrease the incidence of iron-deficiency anemia, decrease dysmenorrhea and decrease the incidence of endometrial and ovarian cancer [5].

COCPs are composed of an estrogens and a progestins. Estrogens involved in combined oral contraceptive pills includes ethinyl estradiol (EE2) or mestranol. There are nine progestins in combined oral contraceptive pills which are norethindrone, norethindrone acetate, ethynodiol diacetate, levonorgestrel, norgestrel, lynestrenol, desogestrel, norgestimate and gestodene [6, 7].

An intrauterine device (IUDs) is a widely used method of birth control with 99% effectiveness. The IUD once inserted in the uterus is immediately effective as a contraceptive [8]. It is the most widely used reversible form of contraception in the world. Copper bearing IUDs is a small, T-shaped device made of polyethylene. The device has two flexible arms that fold down for insertion and expand to form a T shape when released inside the uterus. The copper bearing IUDs protects against pregnancy by reducing motility and viability of sperm, inhibiting ova development and thereby preventing fertilization [9].

Oxidative stress is defined as an imbalance between prooxidants and antioxidants in the cells which is manifested by elevated levels of free radicals [10]. The free radical mediated peroxidation of membrane lipids, increase membrane fluidity and permeability with loss of its integrity that lead to cell damage [11]. Alterations of these structures are associated with the development of several human pathologies including atherosclerosis, cardiovascular disease, cancer, diabetes complications and arthritis [12]. Malondialdehyde (MDA) is the end product of lipid peroxidation which is utilized as a marker of lipid peroxidation in states of elevated oxidative stress [13].

Trace elements (copper, zinc and iron) are essential nutrients for human beings. They are crucial for the functioning of several enzyme systems and are required in a number of metabolic processes in the body. They are involved in gene expression, RNA and DNA metabolism and cellular immune functions. Thus they are of fundamental importance in living organisms [14].

The major lipids present in the plasma are fatty acids, TGs, cholesterol
and phospholipids, are all transported in plasma as lipoprotein particles [chylomicrons (CM), very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL) and high density lipoprotein (HDL)] [15].

**Materials and Methods**

**Subjects**

This study was conducted over a period of 12 months starting from October 2009 till October 2010. All samples collected from Babylon maternity and pediatric hospital in Hilla city. The practical side of the study was performed at the laboratory of biochemistry department in college of medicine / Babylon University.

The general criteria for all subjects in this study includes all women within reproductive age, fertile, with regular menstrual cycle, not suffering from any disease (e.g. Hypertension, diabetes mellitus, asthma etc.), not lactating and not given any medication (e.g. diuretics, steroid, etc.). Any subjects have not this criteria are excluded from this study.

This study includes ninety three healthy women divided into three groups, the first group includes thirty healthy women aged 20-42 year with mean ± SD 32 ± 6.577 year who not used low dose combined oral contraceptive pills or Cu T 380A IUDs (i.e. control group).

**Blood Sampling**

Venous blood samples were drawn from contraceptive users and control subjects by using disposable syringes (5mL) in the sitting position. Five ml of blood was obtained from each subject by vein puncture and pushed slowly into plain disposable tubes. Blood was allowed to clot at 37°C for 10-15 minutes, and then centrifuged at 3000 rpm for approximately 10-15 minutes then the sera were obtained and stored at -20°C until analysis (measure MDA, copper, zinc, iron, total cholesterol, HDL,TG, VLDL and LDL).

**Methods**

Serum MDA concentration are determined by Carl A. Burtis and Edward R. Ashwood procedure [16]. Serum copper concentration and serum zinc concentration are determined by Via melano (Italy) kit. Serum iron concentration are determined by Human (Germany) kit. Serum total cholesterol, TGs and HDL-cholesterol concentration are determined by Biolabo SA (France) kit. VLDL-cholesterol concentration was calculated by dividing triglycerides value by 2.22 [17]. LDL-cholesterol concentration was calculated by using Friedewald equation [18].

**Results**

MDA, copper, zinc, iron, total cholesterol, HDL,TG, VLDL and LDL concentration were measured in sera of thirty women used low dose combined oral contraceptive pills, thirty three women used copper bearing intrauterine devices and thirty healthy women as shown in table (1).
Table 1 Serum MDA, Copper, Zinc, Iron, Total Cholesterol, HDL, TG, VLDL and LDL Concentration in Combined Oral Contraceptive Pills Users, Intrauterine Devices Users and Control Group. [P-value of < 0.05 was considered to be statistically significant].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subjects</th>
<th>No.</th>
<th>Mean ± SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (mmol/L)</td>
<td>COCPs Users</td>
<td>30</td>
<td>4.08 ± 0.72**</td>
<td>3.00-5.54</td>
</tr>
<tr>
<td></td>
<td>IUDs Users</td>
<td>33</td>
<td>2.00 ± 0.41</td>
<td>1.39-2.82</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>1.88 ± 0.49</td>
<td>1.02-3.08</td>
</tr>
<tr>
<td>Copper (μmol/L)</td>
<td>COCPs Users</td>
<td>30</td>
<td>2.47 ± 0.44**</td>
<td>1.45-3.12</td>
</tr>
<tr>
<td></td>
<td>IUDs Users</td>
<td>33</td>
<td>1.61 ± 0.32</td>
<td>1.16-2.53</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>1.58 ± 0.27</td>
<td>0.97-2.10</td>
</tr>
<tr>
<td>Zinc (μmol/L)</td>
<td>COCPs Users</td>
<td>30</td>
<td>8.99 ± 1.45</td>
<td>6.71-13.39</td>
</tr>
<tr>
<td></td>
<td>IUDs Users</td>
<td>33</td>
<td>9.18 ± 1.61</td>
<td>6.41-14.3</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>9.46 ± 1.17</td>
<td>7.33-11.95</td>
</tr>
<tr>
<td>Iron (μmol/L)</td>
<td>COCPs Users</td>
<td>30</td>
<td>17.05 ± 4.66***</td>
<td>8.88-25.57</td>
</tr>
<tr>
<td></td>
<td>IUDs Users</td>
<td>33</td>
<td>9.69 ± 3.97</td>
<td>3.14-19.50</td>
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<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>12.78 ± 3.30</td>
<td>7.24-21.76</td>
</tr>
<tr>
<td>Total Cholesterol (mmol/L)</td>
<td>COCPs Users</td>
<td>30</td>
<td>4.80 ± 0.53**</td>
<td>3.81 – 5.83</td>
</tr>
<tr>
<td></td>
<td>IUDs Users</td>
<td>33</td>
<td>3.77 ± 0.62</td>
<td>2.28 – 4.90</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>4.00 ± 0.54</td>
<td>2.66 – 4.91</td>
</tr>
<tr>
<td>HDL Cholesterol (mmol/L)</td>
<td>COCPs Users</td>
<td>30</td>
<td>0.98 ± 0.24****</td>
<td>0.50 – 1.40</td>
</tr>
<tr>
<td></td>
<td>IUDs Users</td>
<td>33</td>
<td>1.13 ± 0.18</td>
<td>0.80 – 1.56</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>1.17 ± 0.25</td>
<td>0.58 – 1.79</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>COCPs Users</td>
<td>30</td>
<td>1.18 ± 0.39****</td>
<td>0.64 – 2.29</td>
</tr>
<tr>
<td></td>
<td>IUDs Users</td>
<td>33</td>
<td>0.92 ± 0.23</td>
<td>0.56 – 1.61</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>0.97 ± 0.25</td>
<td>0.49 – 1.71</td>
</tr>
<tr>
<td>VLDL-Cholesterol (mmol/L)</td>
<td>COCPs Users</td>
<td>30</td>
<td>0.53 ± 0.17****</td>
<td>0.28 – 1.03</td>
</tr>
<tr>
<td></td>
<td>IUDs Users</td>
<td>33</td>
<td>0.41 ± 0.10</td>
<td>0.25 – 0.72</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>0.43 ± 0.11</td>
<td>0.22 – 0.77</td>
</tr>
<tr>
<td>LDL-Cholesterol (mmol/L)</td>
<td>COCPs Users</td>
<td>30</td>
<td>3.28 ± 0.60**</td>
<td>2.13 – 4.54</td>
</tr>
<tr>
<td></td>
<td>IUDs Users</td>
<td>33</td>
<td>2.22 ± 0.66</td>
<td>0.81 – 3.38</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td>2.39 ± 0.56</td>
<td>1.41 – 3.37</td>
</tr>
</tbody>
</table>

P-value
* COCPs versus Control group (p < 0.001)
** COCPs versus Control group (p < 0.01)
The results in table (1) show significant increase in (MDA, copper, iron, total cholesterol, TGs, VLDL and LDL concentration), non significant decrease in zinc concentration and significant decrease in HDL concentration in sera of women who used COCPs when compared with those of the control group.

Also this study showed significant increase in (MDA, copper, iron, total cholesterol, TGs, VLDL and LDL concentration), non significant decrease in zinc concentration and significant decrease in HDL concentration in sera of women who used COCPs when compared with women who used IUDs group.

The MDA, copper, concentration were found to be non significantly increase, iron concentration significantly decrease and non significantly decrease in (zinc, total cholesterol, HDL, TGs, VLDL and LDL concentration) in sera of women who used IUDs when compared with those of the control group.

Discussion

Lipid peroxidation (MDA)

One of the major findings of the present study was the significant increase in serum MDA concentration observed in the group of women taking COCPs. Some study has shown an increased in serum MDA levels due to the use of estrogen [19]. While other study showed a significant elevation in the lipid peroxide product malondialdehyde (MDA) and decreasing the antioxidant defense represented by decreased glutathion levels in sera of women who used progesterone as a contraceptives when compared with those of the control group [20]. The change in the serum copper concentration is certainly the major event leading to the increased level of malondialdehyde. The results of present study are in agreement with Pincemail J. et al. study [21].

Trace elements (copper, zinc and iron)

An increased concentration of serum copper in COCPs users women due to estrogen. Estrogen-containing combined oral contraceptives caused an increase of plasma copper level that run parallel to that of ceruloplasmin [22]. The estrogen component is mainly responsible for the increased level of serum ceruloplasmin while progesterone causes a less drastic rise [23]. Estrogen acts as an inducer for synthesis of ceruloplasmin RNA templates causing subsequent increase in synthesis of the protein. For this reason the increasing in ceruloplasmin level cause an increase in serum copper concentration [24]. The results of present study are in agreement with Daunter and Elstein study [25].

The decrease in serum zinc concentration may be an important risk factor in oxidant release and the development of DNA damage and cancer, zinc is co-factor in proteins involved in antioxidant defense, electron transport, DNA repair and protein expression [26]. The results of present study are in agreement with Donat D. et al. study [27].

The present study show increase in serum iron level in combined oral contraceptive pills users and it is agree with Behrman R. et al. study [28]. The COCPs can increase the serum iron concentration by reduce the menstrual period to 3-4 day and reduce the amount of blood that loss in each menstrual cycle to 25 mL (35 mL of blood are looses in normal menstrual cycle). COCPs are reducing the endometrial thickness that shorten the
menstrual period. For all these reasons the COCPs can be used in treatment of anemia that results from menorrhagia [29, 30]. Several studies show that the estrogen can increase the hepatic synthesis of transferrin (iron transporter protein) that lead to increase in the serum iron level [31]. The present study also show a decrease in serum iron level in IUDs users and it is agree with Dangour A. et al. study [32]. This decrease in serum iron concentration resulted from increasing in menstrual bleeding (heavier and more prolonged). This increase in menstrual bleeding resulted from either IUDs insertion or endometrial infections [33].

**Lipid profile (Total cholesterol, HDL, TG, VLDL and LDL)**

The effect of oral contraceptives on lipid levels depends on the estrogen dose relative to the progestin dose, in addition to the androgenicity of the progestin. Estrogen tends to have beneficial effects by decreasing LDL-cholesterol concentration and increasing HDL-cholesterol concentration, however, TGs concentration also increase. Progestins seem to have the opposite effect, that is, they cause HDL-cholesterol concentration to decrease and LDL-cholesterol concentration increase [34].

The progestin constituent is demonstrated to provoke serum lipid changes depending on the androgenicity of the progestogen. More androgenic progestogens such as levonorgestrel have larger effects than less androgenic progestogens such as desogestrel and gestodene. Oral contraceptives with ‘second generation’ progestogens (levonorgestrel, lynestrenol and norethisterone) show the most unfavorable effects. The androgenic and antiestrogenic properties of levonorgestrel result in the most pronounced effects. Formulations with levonorgestrel increase total-cholesterol, LDL-cholesterol, and TGs concentration, whereas HDL-cholesterol concentration are decreased [35, 36].

Preparations with other ‘second generation’ progestogens ( lynestrenol and norethisterone) show similar effects on total-cholesterol, LDL cholesterol and TGs concentration. However, the effects on HDL-cholesterol may be more pronounced compared with formulations with levonorgestrel [36, 37].

Blood lipid profile (total cholesterol, HDL, LDL and TGs) is a useful tool in determining risks for cardiovascular diseases. LDL is bad cholesterol being associated in deposition of cholesterol on the walls of arteries and HDL is good cholesterol being associated in carrying cholesterol out of the blood system and is more compact than LDL [38]. Monophasic-phaic COCPs, have been considered to be an important risk factor for dyslipidemia (increase total cholesterol, decrease HDL, increase LDL and increase TGs levels), contributing to the development of atherogenesis and coronary arterial disease [39].

The results of present study are in agreement with Habibollah M. et al. who found a significant increase of total cholesterol, TGs, LDL concentration while HDL concentration significantly decreased in women who used COCPs (ethinyl estradiol and norgesterol) for six months in comparison to the baseline [40].

**Conclusions**

The study concluded that COCPs (Microgynon) cause undesirable effects on oxidative stress, lipid profile and trace elements, in contrast the copper T 380A IUDs observe no undesirable effects on the same
parameters in sera of women those used contraceptives.

References


