Research Article Biochemistry

International Journal of Pharma and Bio Sciences

ISSN
0975-6299

THE EFFECTS OF HORMONAL AND NON-HORMONAL INTRAUTERINE DEVICE CONTRACEPTIVE ON SOME PARAMETERS IN IRAQI WOMEN SERA

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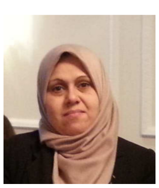
ABSTRACT

This study included eighty healthy women, thirty women used copper bearing intrauterine contraceptive device (IUD), and twenty women used hormonal intrauterine device. Thirty women were used copper bearing intrauterine device (T380A) are group (1), and twenty women were used hormonal intrauterine device (mirena) which release progestin hormone are group (2). Thirty apparently healthy women were taken as a control group who are using male condom contraceptive (group 3), the duration of each contraceptive in these groups for at least 6 months. The mean of age for users of copper bearing IUDs is29.43± 7.92 years, the mean of age for hormonal-IUD (mirena) is 32.63± 8.14 years, and the mean of for the control group is 31.40 ± 9.82 years. Blood samples obtained from Babylon maternity and pediatric hospital in Hilla city and Karbala maternity hospital in Karbala city. The sera obtained from the blood fordeterminingthe effects of copper bearing IUD, and hormonal-IUD on malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase and some trace elements(copper, zinc and iron) concentration. The result of the present research showed significant increase in MDA, SOD and copper concentration,nonsignificant decrease in zinc, significant decrease in iron, nonsignificant increase in BMI, nonsignificant decrease in GSH and catalase concentration in sera of women in group 1, when compared with group 3, also this research showed significant increase in MDA concentration, significant decrease in SOD, GSH, catalase, BMI is significant increase, nonsignificant increase in copper and zinc concentration, and nonsignificant decrease of iron in sera women of group 2 when compared to those of the group 3.

KEYWORDS:intrauterine contraceptive device, hormonal intrauterine device, malondialdehyde (MDA), glutathione (GSH), superoxide dismutase (SOD), catalase and trace elements



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INTRODUCTION

Contraception's is a practice to prevent pregnancy, a contraceptive is a method for that.Intrauterine device (IUD) is an object placed in the uterus to prevent pregnancy [1]. Among modern of IUDs, the two types available are copper-bearing devices, and a hormone-containing device that release a progestin hormone,there are above 10 different kinds of copper IUD presented in different parts of the world, and there is one hormonal device called Mirena [2].

Common types of IUDs available international and which is more using by women are following [3]:

- Inert devices, such as the lippes loop.
- Copper-containing which comprises the copper such as T 380A (T Cu 380A, T Cu 380A with Safe Load, and T Cu 200C), the Multiload (ML Cu 250 and Cu375).

Levonorgestrel-releasing intrauterine system (LNG-IUS) or Mirena, which releasing progestin hormone.

***Copper bearing IUD*:** There are over 10 different kinds of in different parts of the world, the TCu 380 A (ParaGard) for 8 years and the remains for 5 years, U-shaped devices such as multiload 250 and multiload 375 are approved for 5 and 8 years. The Nova-T 380 and Flexi T 380 are approved for 5 years. An IUD is a small plastic and metal object (copper) that is inserted into the uterus using a sterile technique during a pelvic examination. It has anend string that hangs down into the upper part of the vagina to facilitate removal. The surface area of the copper regulates the effectiveness and active life of the device [4].

The Copper T 380A (ParaGard): The main IUD included in this study is the Copper T 380A (ParaGard) or Copper T, which is:

- Widely used.
- Well known for its effectiveness, ease of insertion and removal, varied margin of safety, suitability to clients, and low price.
- Effective for at minimum 12 years [5].

The Copper T 380A was a smaller sized, it looks like the letter T and contains barium sulfate so that it can be seen via X-ray), there are small copper bands on each arm of the T, Copper is released high in the fundus of the uterus, the stem is also wound with copper wire, a thin polyethylene string is close to the stalk for easy removal [4].The mechanism of work of copper-bearing IUDs, such as the copper T, act primarily by preventing fertilization. Copper ions decrease sperm motility and function by changing the uterine and tubal fluid environment, so preventing sperm from reaching the fallopian tube and fertilizing the egg [6].

Hormone releasing intrauterine System (Mirena, LNG-IUD)

Mirena is a hormone-releasing system (Levonogestral intrauterine system) located in the uterus to prevent pregnancy for up to 5 years, it was firstly only licensed as a contraceptive for 3 years but this was extended to 5 years later [7].It was used to treat heavy period for women who choose intrauterine contraception. It is made of soft, flexible plastic as T-shaped and contain a progestin hormone called levonorgestrel, mirena release a small amount (20 µg) of aprogestin hormone directly into the uterus. Mirena has a capsule containing levonorgestral around its stem which release a daily a progestin hormone[8].Mirena may work in some ways. It may thicken the cervical mucus, thin the lining of the uterus, and constrain sperm movement. Mirena may stop release of the egg from the ovary, but this is not the way it works in most cases. Most possible, these activeefforts together to prevent pregnancy. As well as Mirena can cause the menstrual bleeding to be less by diminishing the lining of the uterus [6].

Oxidative Stress:Oxidative stress (OS) is defined asan imbalance between the production of reactive oxygen species (ROS) or reactive nitrogen species (RNS) and antioxidantresistance system which produces the oxidative damage and cause harmful effects [9].OS occurs when oxidants outnumber the available antioxidants. In dissimilarity, reductive stress occurs when antioxidants outnumber the oxidant present in the cell [10].The most well described consequence of free radical attack is lipid peroxidation (LPO) of cell membrane because it is approximatelydefined as the oxidative deterioration of polyunsaturated lipid. LPO of cell membranes results in decreased membrane fluidity, incapability to maintain ionic gradients, and tissue inflammation [11]. The membrane surrounding cells and cell organelles comprises large amounts of polyunsaturated fatty acid (PUFA) side chains, production them susceptible to LPO. The mechanism by which LPO takes place is demonstrated by the formation of malondialdehyde (MDA) from a fatty acid with three double bonds [12].

Antioxidants:Antioxidants are molecules or compounds that scavenge additional free radical to counterthe potential for significant cell damage by excess free radicals. Most antioxidants are electron donors and react with free radicals to form end products such as water. These antioxidants bind and inactivate the free radicals. Consequently, antioxidants defend against oxidative stress and prevent damage to cells [13].Antioxidants can be intracellular or extracellular, enzymatic such as Superoxide dismutase (SOD)*,* Catalase, glutathione reductase (GR) and glutathione peroxidase (GxP), and non-enzymaticantioxidants such as glutathione and vitamin E. Based on these unalike antioxidants are present at a wide range of concentration in body fluids and tissues [13].

Copper:Copper has a number of important functions in the human body, such as copper is required to fix calcium in the bones and to build connective tissue, hair, skin, nails, arteries and veins. Copper must remain in balance with zinc, when imbalances occur, it is disposed to infection. Copper is required for women's fertility and maintain pregnancy, it is important for sexual functions, it has affected men less than women in this area [14].

Zinc: The body contains about 1.5 to 2.5g of zinc, of which the kidneys, pancreas, liver, eyes, prostate and bone contain larger concentrations, however, they do not function as zinc assets, so the body depends on sufficient dietary intake to meet its daily requirements [15].Excessive zinc intake will eventually affect the balance and proper ratios to numerous, which includes iron, calcium, selenium, nickel, phosphorus, copper, as well as vitamin A, B1, and C. It may also reason, or contribute to gastrointestinal problems, hair loss, anemia, loss of libido, prostatitis, ovarian cysts, menstrual problems, reduced immune functions, renal tubular necrosis, dizziness and vomiting . Zinc and copper were studied for a investigated of fertility [16].

Iron:The primary function of iron in the body is the formation of hemoglobin, the essential oxygen-carrying component of the red blood cell (RBC). In combination with protein, iron is carried in the blood to the bone marrow, where with the help of copper, it forms hemoglobin. Iron deficiency is more common during infancy, childhood, adolescence, pregnancy, menstruation, chronic infections, chronic diarrhea, impaired absorption and bleeding [17].

MATERIALS AND METHODS

This study includes three groups, group 1 are thirty healthy females were using copper bearing IUD type T 380A (ParaGard), group 2 are twenty healthy females were using hormonal- IUD , and group 3 are thirty healthy females as a control groupwho are using condoms contraceptive. Users IUDs contraceptives have gotten of Babylon maternity and pediatric Hospital in Hilla city and Karbala maternity Hospital in Karbala city.The duration of the contraceptive using by the women in all groups is at least 6 months. The mean of age for users of copper bearing IUDs is 29.43± 7.92 years, the mean of age for hormonal-IUD is 32.63± 8.14 years, and the mean of for the control groupis31.40 ± 9.82 years, they not smokers with no history of any disease.A questionnaire was designed to obtain the information of the all groups which includes the age, weight, length, duration, any diseases, menstruation status, numbers of birth , breast feeding. For the control group they were the age, weight and length any diseases, menstruation status, numbers of birth, breast feeding. Women suffered from diseases (diabetes mellitus, thyroid diseases, hypertension, cardiovascular diseases, chronic renal failure and malignancy) are excluded, all groups are healthy women and they were married.Lipid peroxidation was evaluated by malondialdehyde (MDA), the level of malondialdehyde was determined by the modified procedure described by Judith B. and Shah S. [18].Glutathione reduced measured according to the principle of 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) is a disulfide chromogen That is readily reduced by sulfahydryl group of GSH to an intensely yellow compound. The absorbance of the reduced chromogen is measured at 412 nm, which is directly proportional to the GSH concentration[19].Cu,Zn-SOD activity to determine by use a sample and rapid method , the principle of this method is based on the competition between the pyrogallol autoxidation by $O_2^{\cdot-}$ and the dismutation of this radical by [20]. Catalas enzyme activity determines according to Go method [21]. Copper, zinc and iron measured according to leflet to kit of Via Melano, Italy.The results were expressed as mean ± SD and analyzed statistically. The differences between the results of IUD user and control were assessed by student's t-test, significant variation was considered when the P value was less than 0.05.

RESULTS

Levels of biochemical parameters of the 30 recruited females who are using copper bearing-IUD (group 1) anda controlgroup who are using male condom user (group 3) aresummarized in Table (1) the results are: insignificant elevation of the BMI in group 1, MDA was a significantly increased (p= 0.045), SOD was a significantly increased (p= 0.044), significant increase of copper (p= 0.021), significant decrease of iron (p= 0.020). However, GSH, catalase and zinc were insignificantly decreased.

Table 1
Biochemical parametersand characteristics of the enrolled user of copper-IUDcontraceptive and control group.

Parameter	Subject	mean± SD	Min-Max	P value
Age (y)	Control IUD user	29.43± 7.92 31.40± 9.82	18.60 – 44.32 19.56 – 45.71	N.S
BMI (Kg/m²)	Control IUD user	25.91± 3.171 26.06 ± 4.32	19.76 -30.41 20.43 – 31.11	N. S
MDI (mmol/L)	Control IUD user	2.14 ± 0.743 2.38± 1.22	1.18 – 3.34 1.66 – 4.25	0.045
SOD (U/L)	Control IUD user	0.104± 0.093 0.165± 0.023	0.056 – 0.152 0.087 – 0.211	0.044
GSH (μM)	Control IUD user	33.24 ± 3.98 31.51 ± 4.31	24.67 – 38.40 19.44 – 35.61	N S
Catalase	Control IUD user	73.70± 12.38 69.41 ±14.22	58.22 – 81.41 44.27 – 76.30	N S
Copper (μmol/l)	Control IUD user	1.94 ±0.48 2.34± 2.62	0.912 – 2.69 1.510 – 3.43	0.021
Zinc (μmol/l)	Control IUD user	15.57± 2.63 14.11 ± 3.51	9.43 – 18.77 8.65 – 16.10	N.S
Iron (μmol/l)	Control IUD user	20.37± 1.63 17.41 ±5.60	12.61 – 26.30 9.17 – 23.60	0.020

p-value versus control group (p≤0.05).
IUD: intrauterine device, BMI: Body mass index, MDA: Malondialdehyde, SOD: superoxide dismutase, GSH: glutathione reduced.

While thelevels of biochemical parameters of the 20 recruited females who are using LNG-IUD (mirena) (group 2) and control groupwho are using male condom contraceptive (group 3) are summarized in Table (2) the results are: Significant elevation of the BMI (p= 0.040), MDA was significantly increased (p= 0.021), SOD was significantly decreased (p= 0.002), significant decrease of GSH (p= 0.001), catalase (p= 0.036), However, copper wasinsignificant increased, zinc and iron were insignificant decreased.

Table (2)

Biochemical parameters and characteristics of the enrolled user of hormonal-IUD contraceptive and control group.

Parameter	Subject	mean± SD	Min-Max	P value
Age (y)	Control IUD user	29.43± 7.92 34.12± 5.66	18.60 – 44.32 18.56 – 45.71	0.035
BMI (Kg/m^2)	Control IUD user	25.91± 3.171 28.54 ± 3.20	19.76 -30.41 21.43 – 36.11	0.040
MDI (mmol/L)	Control IUD user	2.14 ± 0.743 5.5± 1.38	1.18 – 3.34 1.66 – 4.25	0.021
SOD (U/L)	Control IUD user	0.104± 0.093 0.033± 0.026	0.056 – 0.152 0.087 – 0.211	0.002
GSH (μM)	Control IUD user	33.24 ± 3.98 19.63 ±3.17	24.67 – 38.40 19.44 – 35.61	0.001
Catalase (KU/L)	Control IUD user	73.70± 12.38 67.96 ± 2.54	58.22 – 81.41 44.27 – 78.30	0.046
Copper (μmol/l)	Control IUD user	1.94 ±0.48 2.10± 1.31	0.912 – 2.69 1.510 – 3.43	N.S
Zinc (μmol/l)	Control IUD user	14.57± 2.63 14.02 ± 1.38	9.43 – 18.77 8.65 – 16.10	N.S
Iron (μmol/l)	Control IUD user	20.37± 1.63 19.11 ±2.73	12.61 – 26.30 11.17 – 23.60	NS

p-value versus control group (p≤0.05).
IUD: intrauterine device, BMI: Body mass index, MDA: Malondialdehyde, SOD: superoxide dismutase, GSH: glutathione reduced.

DISCUSSION

Trace elements

In this research the levels of serumcopper were significantly higher in group 1 than group 3,while the levels of iron were significantly decreased ingroup 1 when compared with group 3, zinc levels were insignificantly decreased in group 1 than group 3,as shown in Table (1). These results may explain by these devices which contain of copper metal are release of copper ions from copper bearing intrauterine devicesthat is necessary for their contraceptive working [22]. There wereincrease in copper levels among copper bearing IUD users as compared with nonusers [23].The copper ions are absorbed by the endometrial tissue and the copper bearing –IUD (T200A) uses could lead to a 1% increaseof serum copper levels [24]. The using of Copper T380A in women , they were found raised in copper levels about 35% [25]. Copper ions released from an IUD increase the inflammatory response and spread concentrations in the luminal fluids of the genital area that are toxic for spermatozoa [26].

It is also important to note that the patients with special risk groups such as Wilson's disease patients could be using the T380 [27].Zinc reduction, while copper increased in the females who are using copper-IUD [28]. The features deceasing zinc absorption embrace presence of copper at high levels it since of copper must remain in balance with zinc. [14]. Iron levels were decreased in group 1 than group 2 ,this result may be due to the heavy menstrual cycle in users of copper-IUD, the greatest common copper-IUD related side effect is excessive uterine bleeding. The menstrual blood loss is commonlydoubled after the insertion of a copper IUD especiallythrough the first 3 to 6 months of use, frequently; the menstrual blood may be excessive to the degree of causing iron deficiency anemia, with the phase of increased bleeding and pain [29]. In this study copper levels were insignificantly increased, zinc and iron levels were insignificantly decreased in group 2 which are using mirena when compared with group 3 (control group) as shown in Table (2), these results trend to the using of this contraceptive (mirena) which release of progestin only was not effect on the copper and zinc levels, while combined contraceptive which comprise of estrogen and progestin were effected of these elements Estrogen-containing combined oral contraceptives caused an increase of plasma copper level that run equivalent to that of ceruloplasmin [30]. Zinc is a co-factor in proteins involved in antioxidant defense and it decrease when the oxidative stress increase [31].Using of Mirena may experience of menstrual period this occur with 2 out of 10 women stop having period after 1 year of Mirena use, the period retains when mirena is removed. The period may become irregular and it may have bleeding and spotting between menstrual period, especiallythrough the first 3 to 6 months. A few women have heavy bleeding during this time. A period, usuallybecome lighter and the number of bleeding days is possible to decrease, but may remain irregular, or it may level find that period stop [32].

Lipid peroxidation (MDA)and antioxidants

In the present study MDA levels were significantly higher in group 1 than group 3 , SOD levels were significantly increased in group 1 than group 3, GSH was also insignificantly decreased,CAT levels were insignificantly decreased, shown in Table(1). These results can be explainedby the effects of copper-IUD on oxidative stress,whereas OSwas increased in the women who are using copper bearing-IUD (T380A) [33]. In mechanism of copper-bearing IUDs, it was released copper ions to decrease sperm motility and function by altering the uterine and tubal fluid environment [6]. Copper ions have a role in the generation of free radicals and OS describe oxidative damage to DNA and lipid peroxidation as the chief effects of oxidative stress, copper has been found to bind to non-histone nuclear matrix proteins as sites where DNA loops anchor, signifying that the electron-rich DNA strength be a target of copper interceded oxidative damage [34, 35]. Mixture of Cu^{+2} and H_2O_2producedcomponent scission and alteration of the bases in DNA, while H_2O_2 or Cu^{+2} alone did not cause any of this damage, it has been optional that Cu^{+2} ions bind to DNA at guanine sites, where it reacts with H_2O_2 and a reducing agent to create $OH^{\bullet}$ which attacks the DNA bases in a site-specific manner [36].]. Superoxide dismutase Cu,Zn-SOD, as endogenous antioxidant, its supposed to apply both an antioxidant and pro-oxidant function is the copper-binding enzyme Cu,Zn-SOD, which catalysis the dismutation of two $O_2^{\bullet-}$ ions into O_2 and H_2O_2 [37]. Using its individual dismutation product, H_2O_2 as a substrate, the enzyme can make $OH^{\bullet}$ by a Fenton-like reaction involving its bound copper ions [38]. Increased of superoxide radical formation in user of Copper bearing IUD [39]. The $OH^{\bullet}$ may react with the Cu,Zn-SOD molecule itself or with other molecules in the neighborhood of its generation site. If $OH^{\bullet}$ reacts directly with Cu,Zn-SOD, copper ions can be released from the damaged enzyme, which in go can enhance the Fenton reaction by reacting with H_2O_2 [40]. bound Cu^{+2} - $OH^{\bullet}$rather than free $OH^{\bullet}$ is generated through the reaction of intact

Cu,Zn-SOD with H_2O_2. This Cu^{+2} -$OH^{\bullet}$ intermediate may be accountable for the inactivation of Cu,Zn-SOD by being scavenged intramolecularly, thus producing obliteration of ligands for Cu^{+2} in Cu,Zn-SOD and fragmentation of SOD [41].GSH as an antioxidant and it decreased when OS increased [42], so, it decreases in users of copper-IUD.Catalase (CAT) is one of the antioxidant enzyme, decomposes hydrogen peroxide to water and oxygen. CAT role in oxidative stress as an antioxidant and connected diseases has been extensively studied [43]. In this studyoxidative stress (MDA) levels were significantly higher for group 2when compared with group 3,The levels of SOD were significantly decreased in group 2 than group 3,GSH levels were also significantly decreased in group 2, CAT levels were significantly decreased,as shown in Table (2). These results may be explained by the effects of levonogestrel-releasing intrauterine device on oxidative stress, the OS was increased in women who are using of mirena[23, 44].Progestin-induced endometrial bleeding is characterized by endometrial stromal penetration of leukocytes and these cells canister release superoxide ions [45]. In provision of this finding, the productions of epoxides lead to rise the release of oxygen radicals which in turn can react with polyunsaturated fatty acids in cell membrane.Therewere an increase of epoxide synthesis, which connected with vasodilatation and rupture of blood vessels in the endometrium [46, 47]. Cu,Zn-SOD is an antioxidant and it is decreased when the OS increased [48] therefore, it was decreased in women who are using progestin only as contraceptive SOD is the major resistance upon superoxide radicals and is the first defense line against oxidative stress. Different forms of SOD are present in the body, manganese is a cofactor of Mn-SOD form, Cu, Zn-SOD another form of SOD, copper and zinc are cofactor of it.Glutathione (GSH) is an endogenous compound using a double role in the antioxidant against oxidative stress and detoxification of xenobiotic. GSH levels were decreased in several types of diseases such as breast cancer, bladder cancer, ovary cancer, placenta cancer and acute myocardial infarction [49].Catalase (CAT) is present in every cell and in specific in peroxysomes, cell structures that use oxygen in direction to detoxify toxic substances and produce H_2O_2 [43]. Catalase convertsH_2O_2 into water and oxygen.

Body mass index (BMI)

In this research body mass index levels were insignificantly increased in group 1 than group 3, while BMI levels were significantlyincreasedin group 2 than group 3, it calculated from the following equation:

BMI = Weight (Kg) /Height (m^2)

The copper-IUD and barrier contraceptives methods has not been related to a change in body weight [50]. Copper-IUD have been decreased the of endometrial hyperplasia and cancer [51]. The copper IUD is perhaps the best contraceptive option for obese women [52]. ABMI of females who used mirena or (LNG-IUD) showed a fast decrease of efficacy with increasing body mass index (BMI) [53]. The levonogestrel-releasing intrauterine device, in long-term users, has been associated with a lesser increase in weight that is alike to the weight gain associated with increasing age [54]. LNG showed a rapid decrease of efficacy with increasing body mass index (BMI), reaching the point where it appeared no different from pregnancy rates [55].

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