Oxidative Stress and Some Related Trace Elements in Women with Unexplained Infertility

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Abstract

This study aims to evaluate the oxidative stress and related trace elements (iron and copper) in women with unexplained infertility (UI). 60 women with unexplained infertility as well as (40) women with polycystic ovarian syndrome (PCOS) aged (20-35) year were included in this study. The women were divided according the type of infertility into primary and secondary groups (1ºUI, 2ºUI, 1ºPCO and2ºPCO) respectively. 50 healthy fertile women with the same age were included in this study as control group. The results showed a significant increase in serum malondialdehyde (MDA) levels in all infertility groups compared to control group. Whereas serum levels of the antioxidants glutathione (GSH), superoxide dismutase (SOD) and ceruloplasmin (Cp) were significantly decreased in all infertility groups in comparison with control group. Serum levels of iron and copper significantly increase in unexplained infertility groups in comparison with explained infertility and control groups. No significant differences in iron levels between explained infertility groups and control. Whereas serum levels of the copper were significantly increased in explained infertility groups in comparison with control group. This study concluded that is a disturbance in serum oxidant- antioxidant balance in women with UI and PCOS infertility.

Keywords: Unexplained infertility, Lipid per oxidation, Ceruloplasmin, Superoxide dismutase Iron, Copper.

Introduction

Infertility is defined as the inability to conceive naturally after one year of regular unprotected intercourse. Most of the time, infertility is some degree of sub fertility in which 1 in 7 couples need specialist help to conceive. Sub fertility can be either primary or secondary.1Infertility of unknown origin comprises both idiopathic and unexplained infertility.2,3

Most of the infertile couples have one of these three major causes including a male factor, ovulatory dysfunction, or tubal-peritoneal disease.4According to the Center of Disease Control the causes of female infertility can be divided into three broad categories including defective ovulation, transport and implantation.5 Unexplained infertility is one of the controversial subjects in infertility on which agreement is rarely found among practitioners. It is a term used to define 30–40 % of couples in whom standard investigations including semen analysis, tests of ovulation and tubal patency have failed to detect any gross abnormality.6 Couples with unexplained infertility suffer from both diminished and delayed fecundity.5 The diagnosis of unexplained infertility may be frustrating because if there is no explanation for infertility, there is no effective treatment6. The prognosis is worse if the duration of infertility exceeds 3 years and female partner is >35 years of age.7 Treatment has been indicated if the duration is more than 2 years or the female partner is > 35 years of age.7, 8 Polycystic ovary syndrome (PCOS) is characterized by hyperandrogenism, chronic an ovulation and polycystic ovaries.9, 10

The prevalence of classical forms of PCOS is from 6-9 to 19.9% and is even higher in women with menstrual disorders (17.4-46.4%), hyperandrogenism (72.1-82.0%), and anovulatory infertility (55-91%).11,12 Clinical symptoms include ovulatory dysfunction, hyperandrogenism, and polycystic ovaries. Metabolic disorders (obesity, insulin resistance, impaired glucose tolerance, type II diabetes mellitus, and dyslipidemia) are
Generation of reactive oxygen species (ROS) is a normal feature of basal aerobic metabolism that supports life. They are active derivatives produced during the intermediate steps of oxygen reduction, which are catalyzed by small molecules such as iron and copper. Biological systems contain an abundant amount of O₂. Free radicals are often generated from O₂ and partially from normal metabolic processes in the body.

They are unstable and highly reactive due to unpaired electrons that are capable of initiating an uncontrolled cascade of chain reactions, resulting in cellular damage and disease. ROS are involved in physiological functions in female reproduction such as oocyte maturation, ovarian steroidogenesis, ovulation, implantation, and formation of the fluid-filled cavity, blastocyst, and luteolysis and luteal maintenance in pregnancy. ROS acts as mediators of various signaling pathways.

Elevated or sustained generation of free radicals lead to imbalance in the intracellular redox homeostasis. Excess levels of free radicals and ROS can be neutralized by antioxidants. Any imbalance between ROS and antioxidants can cause oxidative stress. Oxidative stress involved in various pathologies of female reproductive tract like polycystic ovarian syndrome, endometriosis, tubal factor infertility, unexplained infertility, fibroids, recurrent pregnancy loss, spontaneous abortions. Lipid peroxidation is a self-propagating reaction unless it is counteracted by antioxidants.

Malondialdehyde (MDA) is a l membrane. As hypoxia intensifies the peroxidation and cell membrane disruption, increase in extra cellular activity of Lactate dehydrogenase (LDH). LDH can be used as a hypoxia marker. Follicular fluid contains high concentrations of anti-oxidants, which protects oocytes from ROS-induced damage. An imbalance in the pro-and anti-oxidant systems in the follicular fluid could lead to abnormal development of the oocytes and impaired fertility. Under normal conditions, scavenging molecules known as antioxidants convert ROS to H₂O₂ to prevent overproduction of ROS. There are two types of antioxidants in the human body: enzymatic antioxidants and non-enzymatic antioxidants. Enzymatic antioxidants are also known as natural antioxidants; they neutralize excessive ROS and prevent it from damaging the cellular structure. Enzymatic antioxidants are composed of superoxide dismutase, catalase, glutathione peroxidases, and glutathione reductase, which also causes reduction of hydrogen peroxide to water and alcohol. Non-enzymatic antioxidants are also known as synthetic antioxidants or dietary supplements. The body's complex antioxidant system is influenced by dietary intake of antioxidant vitamins and minerals such as vitamin C, vitamin E, selenium, zinc, taurine, hypotaurine, glutathione, beta carotene, and carotene.

Glutathione is a biologically dispersed, pivotal antioxidant counterpart which is categorized under the thiol family, and directly interacts with H₂O₂ to reduce it to biochemically stable 110 forms of H₂O and O₂. Deeper insights into the biochemical mechanism show that the reduced form of GSH reacts with H₂O₂ via an enzymatic process catalyzed by glutathione peroxidase. Once the reaction is in progress, the available GSH is converted into the oxidized form of glutathione (GSSG). Therefore, continuity of these processes on the activity of superoxide dismutase (SOD). Glutathione is present in the oocyte and tubal fluid and has a role in improving the development of the zygote beyond the 2-cell block to the morula or the blastocyst stage.

The glutathione oxidation–reduction ratio is a good marker of both oxidative stress and antioxidant status. SOD enzymes are a family of metalloenzymes, found in virtually every oxygen-based organism, and their major function is to catalyze the dismutation of O₂⁻ to H₂O₂. In humans, SOD occurs in high concentrations in brain, liver, heart, erythrocytes, kidney, and spleen. Corpus luteum is produced after ovulation and ROS are also produced in the corpus luteum. Cu, Zn-SOD decreases and ROS increases during the regression of corpus luteum.

This activity parallels the change in progesterone levels. Complete disruption of the corpus luteum causes a substantial decrease of Mn-SOD in the regressed cell and cell death is imminent. Cu, Zn-SOD is related to progesterone production and Mn-SOD protects luteal cells from oxidative stress.
There are also some other oxidative stress markers such as superoxide dismutase, Cu-Zn superoxide dismutase, Mn superoxide dismutase, glutathione peroxidase, γ-glutamyl synthetase, and lipid peroxides ovarian physiology. \(^{31,32}\) Cp is one of the best examples of a multifunctional protein. Indeed, Bielli and Calabrese have dubbed it a “moonlighting protein”, and for good reason.\(^{33}\) All the established and documented functions of Cp are based on the fact that it contains and carries copper.\(^{34}\)

Whether the almost equally abundantapo form in the plasma plays some kind of independent role in mammalian metabolism (or copper metabolism) has not been explored.\(^{35}\) Cp was shown to inhibit a variety of oxidative reactions, involving peroxide and superoxide, including the Fenton reaction that forms OH radicals from \(\text{H}_2\text{O}_2\), and dismutation of superoxide.\(^{36}\) Trace elements play a vital role in many different biological functions. The relationship of trace elements to different disease states has long been known.\(^{37}\)

Although trace elements are essential components of biological structures or essential for enzymatic function, they can be toxic at higher concentrations beyond what is necessary for their biological functions.\(^{38}\) Nutritional elements such as Zn, Cu, and Se are components of proteins, enzymes and hormones, which regulate many processes, including balancing redox reactions, immune response, and formation of connective tissue during development.\(^{39}\) Iron is an element essential for life and maintaining normal organism function. It is also necessary for the production of myoglobin, oxidative phosphorylation within mitochondria, DNA synthesis.\(^{40}\) Copper is normally bound to proteins as an essential enzymatic component. In terms of storage capacity, high concentrations of Cu are found in liver, which is the central organ for Cu homeostasis.\(^{41}\)

Other organs that have high concentrations of Cu are the brain, heart, stomach and intestine. Free Cu\(^{+}\) is a potent oxidant causing the generation of ROS in cells. Thus, the tight regulation of Cu homeostasis is required to maintain Cu uptake, transport, storage, and excretion activities.\(^{42}\)

**Experimental**

This study has been conducted at AL-Hussein Teaching Hospital in Thi-Qar, Biochemistry Laboratory in College of Science, at the period between 10/8/2016 to 25/5/2017. Informed consent was obtained verbally from all participants. A total of one hundred women with infertility of the ages 20 – 35 years. The women are already diagnosed as infertile women by the consultant medical staff, according to clinical examination and symptoms.

Sixty women suffer from unexplained infertility whereas the forty women suffered from polycystic ovarian syndrome to get the normal values of studied parameters, the study included fifty healthy age matched fertile women with a history of at least one child birth were also enrolled. The details of the numbers and age of the two groups are illustrated in Table I

<table>
<thead>
<tr>
<th>Groups</th>
<th>No</th>
<th>Rang of Age (year)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (infertile women)</td>
<td>100</td>
<td>20-35</td>
</tr>
<tr>
<td>Control (fertile women)</td>
<td>50</td>
<td>20-35</td>
</tr>
<tr>
<td>Total</td>
<td>150</td>
<td>20-35</td>
</tr>
</tbody>
</table>

Also the infertile women that included in this study were divided into subgroups according to the type of infertility of infertile women showed as followings:

(1ºUI): included (30) woman with primary unexplained infertile women.

(2º UI): included (30) woman with secondary unexplained infertile women.

(1ºPCO): included (20) woman with primary explained infertile women (PCOS).

(2ºPCO): included (20) woman with secondary explained infertile women (PCOS).

**Collection of Blood Samples**

About (5mL) of blood samples was collected by vein puncture using a sterile disposable syringe in plain plastic tubes. The serum was separated immediately in order to allow clotting at room temperature. The blood was centrifuged at 3000 revolution per minute (rpm) for 10 minutes and stored in plain
tubes at (-20°C) until used or immediately analyzed.

**Determination of Serum Malondialdehyde (MDA)**

The level of serum malondialdehyde was determined spectrophotometrically according to the method of Muslih et al., 2002.\(^{43}\) In brief; to 150 μl serum sample the following was added: 1ml of 17.5% trichloroacetic acid TCA provided by (BDH, England ), and 1ml of 0.66% TBA provided by (BDH, England) which is prepared by dissolving 39.6 mg of DTNB into 10 ml of the phosphate buffer solution PH 7. The result solution was kept for 60 seconds at the room temperature. The equivalent solution (Blank) was prepared as the steps 1 and 2. The absorbance of the result solution was measured by using optical spectrum at the wavelength of 420 nm. The total concentration of the Glutathione was calculated as:

\[
GSH \left( \frac{\mu mol}{L} \right) = \frac{(T - B) \times D}{\varepsilon} \times 1000
\]

T: the absorbance of the solution.
B: the absorbance of the equivalent solution (Blank).
D: the dilute factor.
\(\varepsilon\): Molar extension coefficient equal to 13600 mol\(^{-1}\)L cm\(^{-1}\)

**Determination of Serum Glutathione (GSH)**

Serum glutathione concentration was measured according to Ell man’s method.\(^{44}\) The level of serum GSH was determined spectrophotometrically with 0.02 ml of the plasma was added to 9 ml of water and added 1ml of phosphate buffer solution PH 8 to the solution. The previous solution was mixed 3 ml of with 0.02 ml of DTNB solution ((5, 5-dithio-bis (2-Nitrobenzoic acid) provided by BDH England which is prepared by dissolving 39.6 mg of DTNB into 10 ml of the phosphate buffer solution PH 7). The result solution was kept for 60 seconds at the room temperature. The equivalent solution (Blank) was prepared as the steps 1 and 2. The absorbance of the result solution was measured by using optical spectrum at the wavelength of 420 nm. The total concentration of the Glutathione was calculated as:

\[
GSH \left( \frac{\mu mol}{L} \right) = \frac{(T - B) \times D}{\varepsilon} \times 1000
\]

T: the absorbance of the solution.
B: the absorbance of the equivalent solution (Blank).
D: the dilute factor.
\(\varepsilon\): Molar extension coefficient equal to 13600 mol\(^{-1}\)L cm\(^{-1}\)

**Determination of Serum superoxide dismutase (SOD)**

Serum concentration superoxide dismutase (SOD) was measured according to the method of Superoxide Dismutase Micro plate Assay Kit provided by Cohesion Biosciences USA

**Determination of Serum Ceruloplasmin (Cp)**

Serum Cp concentration was measured according to the method of Menden et al. 1997. 4550 mg of PPD provided by BDH, England was dissolved in 5 ml(4 ml of distilled water (D.W) and 1 mL of glacial acetic acid). 8.15gm sodium acetate hydrate provided by BDH, England was dissolved in 30 ml of D.W then added to first solution, mixed and completed the volume to 50 mol with distill water. 100 mg of sodium azide provided by Reideldehain, Germany was dissolved in (500 ml) of D.W then was kept cold in a refrigerator prior to use. One mL of the first solution was pipetted into glass test tubes, incubated at 37oC for 1 min then(0.1 mL) of serum was added, incubated at 37oC for 15 min, then tubes were removed and placed in iced-bath for 30 min five ml of cold sodium azide solution was added, mixed under 25oC in water bath. The absorbance of test and blank was read in spectrophotometer at 525 nm. The concentration of Cp was calculated as:

\[
Cp \left( \frac{mg}{L} \right) = \frac{T - B}{\varepsilon} \times 10
\]

\(\varepsilon\) = the extinction coefficient of Cp equal to 0.68
Determination of Serum Iron and Copper

Serum iron concentration was measured according to the method of iron kit. The used reagents were supplied by (Randox, UK). Serum Cu concentration was measured according to the method of copper kit (Spectrum, Germany).

Statistical Analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage and least significant difference – LSD test was used to significant compare between means in this study.

Result and Discussion

The women with primary and secondary unexplained infertility (UI) had the mean age of 29.25 ± 6.26 and 33.69 ±6.36 years respectively, and the mean age of women with primary and secondary explained infertility (PCOS) groups were 25.844 ± 4.47 and 28.44± 4.21 years respectively. The mean age of the control group was 32.28±6.70 years. The women with primary and secondary (UI) had the mean BMI values of 26.44±3.24 and 27.27±4.04 kg/m² respectively, and the mean BMI values of women with primary and secondary (PCOS) were 30.77±3.11 and 31.34±4.12 kg/m² respectively. The mean BMI value of control group was 64.02 ± 6.96 kg/m².

Serum Malondialdehyde Concentration

The results of Table (II) showed a significant increase in concentration of serum MDA in all women with unexplained infertility (UI) and explained infertility (PCO) in comparison with control groups (p≤ 0.05). The same Table shows a significant increase in concentration of serum MDA in two groups of woman with explained infertility (1ºPCO) and (2ºPCO) in comparison with two groups of women with unexplained infertility (1ºUI) and (2ºUI) respectively (p≤0.05). However, there were no significant differences in concentration of serum MDA between (1ºUI) and (2ºUI) (p≥0.05) and no significant difference in concentration of serum MDA between (1ºPCO) and (2ºPCO) (p≥0.05). The results showed increase in concentration of serum MDA in the women with unexplained infertility. These results are in agreement with studies of Savita et al. 2009; Al.Mukhtar et al. 2012 and ALL-Ahmed et al. 2015. In the human body, reactive oxygen species (ROS) are formed under physiologic and pathologic condition. They can be produced from endogenous sources, for instance during aerobic metabolism and due to different metabolic pathways, or as part of defense mechanism of the body. In addition, ROS can be formed exogenously as a result of numerous environmental pollutants and by cigarette and alcohol use. Data have been implicated that regulated levels of ROS in ovaries, endometrium, fallopian tube, embryo, and peritoneal fluid play a role in tissue remodeling, hormone signaling, ovarian steroidogenesis, folliculogenesis, maturation of oocyte, tubal function, and cyclical and endometrial changes. The high rate of free radicals production in female with unexplained infertility may be generated from increases metabolism and depletion of protective antioxidants. The increase in concentration of serum MDA in the two groups of women with explained infertility in comparison with fertile women. These results agree with the result of Deepika et al. 2014; Sumithra et al. 2015 and Zahoorunnisa et al. 2017. Also there was a significant increase in concentration of serum MDA in the two groups of woman with explained infertility (1ºPCOS) and (2ºPCOS) in comparison with the two groups of the woman with unexplained infertility (1ºUI) and (2ºUI) respectively (p≤0.05). These results agree with the results reported by Diamond et al., 2017. ROS level have been shown to be positively associated with obesity, hyperinsulinemia, androgen excess, chronic inflammation. Obesity is one factor leading to the more serious oxidative status of PCOS, also Insulin resistance (IR) encourages oxidation stress because hyperglycemia and higher levels of free fatty acid lead to reactive oxygen species (ROS) production. When excess glucose or free fatty acid are absorbed in the cell, a large number of reducing metabolites, just like pyruvic acid and acetyl coenzyme A, will be transferred into mitochondria for oxidization, leading to enhancing the activity of electron transport chain and single electron transfer, finally resulting in increasing ROS production.

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the lipid per oxidation in women with PCOS evaluated in comparison with women with UI may be the cause due to effects of Obesity, hyper androgenemia and Insulin resistance.

In the same Table there are no significant differences in concentration of serum ceruloplasmin between (1ºUI) and (2ºUI) as well as (1º PCO) and (2º PCO) respectively groups (p≤0.05), can be observed. This decrease in concentration of serum GSH and SOD in two groups of women with (UI) agrees with the studies of Al Mukhtar et al., 2012 and ALL-Ahmed et al., 2015.47,48

Also the decrease in concentration of serum GSH and SOD in two groups of women with (PCOS) agrees with the studies of Kurdoglu et al., 2012;Murri et al., 2013 and Santos et al., 2016.59,60,61 Elevated ROS levels in patients with unexplained infertility implies exhausted antioxidant defense, resulting in the inability to scavenge ROS and neutralize their toxic effects.62Combelles et al., 2009 and Victor et al., 2011 suggested that found positive relationship between excess oxidative stress an decrease the antioxidant enzymes activities which lead to impaired infertility in females.63,64

These results are in agreement with the results obtained from this project. The low serum ceruloplasmin levels in women with unexplained infertility supports the hypothesis that low antioxidant levels are linked to the path physiological aspects of unexplained infertility. Ceruloplasmin, beside its prooxidant activity 65 behaves as a potent antioxidant for various oxygen radicals collecting oxygen derived free radicals in the blood stream.65, 66 Furthermore the low level of serum ceruloplasmin in infertile women may be due to its role in the ferroxidase activity which is of greatest importance as it converts reduced (ferrous) iron linked with ferritin. Fe2+ acts as pro-oxidant agent because of its readiness to change from one valency state to another.

Table II: Serum MDA levels of control and patients groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO</th>
<th>MDA(μmol/l) means SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>3.88 ± 0.99 *</td>
</tr>
<tr>
<td>1º UI</td>
<td>30</td>
<td>3.06 ± 0.36 b</td>
</tr>
<tr>
<td>2º UI</td>
<td>30</td>
<td>3.11± 0.32 b</td>
</tr>
<tr>
<td>1º PCO</td>
<td>20</td>
<td>3.56 ± 0.97 *</td>
</tr>
<tr>
<td>2º PCO</td>
<td>20</td>
<td>3.06 ± 0.36 b</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>0.34</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.D values with non-identical superscript (a, b or c ...etc.) were considered significantly differences (P ≤ 0.05).Control: fertile woman; 1º UI: Primary unexplained infertility; 2º UI: Secondary unexplained infertility; 1º PCO: Primary explained infertility; 2º PCO: Secondary explained infertility; No: Number of subjects; SD: Standard deviation, LSD: Least Significant Difference.

Serum Antioxidant Concentrations

Table (III) shows a significant decrease in concentration of serum GSH in two groups (1ºUI) and (2ºUI) in comparison with control group (p<0.05). Also it is found a significant decrease in concentration of serum GSH in (2ºUI) in comparison with (1º UI), (1º PCO) and (2º PCO) respectively (p≤0.05). The results show a significant decrease in concentration of serum GSH in two groups of women with PCO in comparison with control group (p≤0.05). However there are no significant differences in concentration of serum GSH between (1º UI), (1º PCO) and (2º PCO) respectively (p≤0.05).

The same Table shows a significant decrease in concentration of serum (SOD) in (1ºUI) and (2ºUI) in comparison with control groups (p≤0.05). Also it is found a significant decrease in concentration of serum (SOD) in (1º PCO) and (2º PCO) groups in comparison with control group (p≤0.05). However there are significant differences in concentration of serum (SOD) between (1ºUI) and (2ºUI) as well as (1º PCO) and (2º PCO) respectively groups (p≤0.05).

The results show a significant decrease in concentration of serum ceruloplasmin in all groups of the women with unexplained infertility (UI) and explained infertility (PCOS) in comparison with the control (p≤0.05).
Table III: Serum antioxidant levels of control and fertile women groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO.</th>
<th>GSH (µmol/L) mean± SD</th>
<th>SOD (U/mg) mean± SD</th>
<th>CP (g/I) mean± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>558.36±114.07 a</td>
<td>6.08±0.81</td>
<td>3.78 ± 0.70</td>
</tr>
<tr>
<td>1º UI</td>
<td>30</td>
<td>309.42±73.94 b</td>
<td>3.46±0.36 b</td>
<td>2.91 ± 0.87</td>
</tr>
<tr>
<td>2º UI</td>
<td>30</td>
<td>255.53±86.16 b</td>
<td>3.95±0.50 b</td>
<td>3.05 ± 0.78</td>
</tr>
<tr>
<td>1ºPCO</td>
<td>20</td>
<td>285.83±89.41 b</td>
<td>3.72±0.61</td>
<td>1.45±0.39 c</td>
</tr>
<tr>
<td>2º PCO</td>
<td>20</td>
<td>276.66±84.14 b</td>
<td>3.47±0.54</td>
<td>1.37±0.34 c</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>37.97</td>
<td>0.77</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Legend as in Table II

Serum Trace Elements Concentration

Table (IV) shows a significant increase in the concentration of serum Fe in the two groups of the women with unexplained infertility (1ºUI) and (2ºUI) in comparison with control group (p≤0.05). Also, it is found a significant increase in the concentration of serum Fe in (1ºUI) in comparison with (2ºUI), (1ºPCOS) and (2ºPCO) groups respectively (p≤0.05), but there are no significant differences in the concentration of serum Fe between (2ºUI), (1ºPCOS) and (2ºPCO) groups respectively (p≤0.05). The same Table shows no significant differences in the concentration of serum Fe in the two groups of women with explained infertility (1ºPCOS) and (2ºPCO) in comparison with control group (p≤0.05).

The same Table shows a significant increase in the concentration of serum Cu in the two groups of the women with unexplained infertility (1º UI) and (2º UI) in comparison with control group (p≤0.05). Also it is a significant increase in the concentration of serum Cu in the two groups of women with unexplained infertility (1ºUI) and (2ºUI) in comparison with two groups of the women with explained infertility (1ºPCO) and (2ºPCO) respectively (p≤0.05). While there are no significant differences in the concentration of serum Cu between the two groups of women with unexplained infertility (p≤0.05).

The results shows a significant increase in the concentration of serum Cu in the two groups of the women with explained infertility (1ºPCO) and (2ºPCO) groups in comparison with the control group (p≤0.05). But no significant differences in the concentration of serum Cu (1ºPCO) and (2ºPCO) groups (p≤0.05), can be observed. This study shows a significant increase in the concentration of serum Fe in two groups of women with unexplained infertility (1ºUI) and (2ºUI) in comparison with fertile women.

Also, there are a significant increase in the levels of serum Fe in (1ºUI) in comparison (2ºUI), (1ºPCOS) and (2ºPCO) groups respectively (p≤0.05). This result disagreement with the results study of Alwais et al., 2016. There are no significant differences in the concentration of serum Fe between (1ºPCOS) and (2ºPCO) in comparison with fertile women. This result matched with the results study of Alwais et al., 2016.

The non-significant differences in the concentration of serum Fe between two groups of women with explained infertility (1ºPCOS) and (2ºPCO) in comparison with fertile women. This result matched with the results of the studies of Li et al., 2016 and Rashid et al., 2017. In this study it is found high concentration of serum iron in women with unexplained infertility. There are few studies investigated the effect of iron overload (toxicity) and fertility and even less with regard to female fertility. However, there is a link between iron overload and female infertility.

Excess iron leads to reduced production of the hormones LH and FSH from the anterior pituitary suggesting impaired oocyte maturation and low ovarian reserve. In this study, no significant differences in serum levels of iron and were observed between the two groups of the women with (PCOS) and control group. A research conducted by Taghavi et al. in 2009 in Iran on 50 PCOS patients without overweight did not show iron overload in these patients. A cross-sectional study of Li et al. on 578 people in 2016 indicated that the serum levels of iron had no difference between the two PCOS and non-PCOS groups.

In this study the high concentration of serum Cu in the women with unexplained infertility reported by the current study matched with the results of the study of AL-Saraf et
al., but disagreement with results of study of Bawa and Tyagi who suggest the copper lower in the women with primary and secondary unexplained infertility than in control subjects and suggest that hypocupraemia may be a factor in the etiology of infertility in these women.

Whereas the reported significant increase in the concentration of serum Cu in the two groups of women with explained infertility in comparison with fertile women, matched with the results of the studies of Fenkci et al. 2003; Kurdoglu et al. 2012 and Li et al. 2016, but disagreement with the results of the study of Bawa and Tyagi who suggest the copper lower in the women with primary and secondary explained infertility than in control subjects.

The elevation in serum copper levels may directly affect infertility rates by lowering progesterone levels resulting in an ovulation, implantation failure or luteal phase deficits. Copper has also been shown to block the absorption of many essential minerals directly involved with reproductive pathways, especially zinc. In this work there were high levels of serum Cu concentration in women with PCOS in comparison with fertile women. Also Kurdoglu et al. suggested the PCOS patients also had a statistically and significantly higher amount of copper than the control group.

The survey conducted by Celik on copper, homocysteine, and early vascular disease in lean women with PCOS showed high copper levels in PCOS patients. A similar result was obtained by a study on mice by Chen et al., 2015. Previous studies have shown that patients with PCOS Copper is involved in the metabolism of oxygen and plays an important role in free radical reactions. This study concluded that is a disturbance in serum oxidant- antioxidant balance in women with UI and PCOS infertility.

### Table IV: Serum trace elements levels of control and infertile groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>NO.</th>
<th>Fe (mg/dl) mean± SD</th>
<th>Cu (mg/dl) mean± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>50</td>
<td>82.55± 15.79 *</td>
<td>105.11±21.38 *</td>
</tr>
<tr>
<td>1º UI</td>
<td>30</td>
<td>125.01 ± 24.28 *</td>
<td>188.06±56.40 *</td>
</tr>
<tr>
<td>2º UI</td>
<td>30</td>
<td>114.69 ±26.36 *</td>
<td>175.24±43.00 *</td>
</tr>
<tr>
<td>1º PCOS</td>
<td>20</td>
<td>84.63 ±5.65 *</td>
<td>121.41±17.52 b</td>
</tr>
<tr>
<td>2º PCOS</td>
<td>20</td>
<td>84.30 ±7.64 c</td>
<td>120.15±15.93 b</td>
</tr>
<tr>
<td>LSD</td>
<td></td>
<td>7.66</td>
<td>14.43</td>
</tr>
</tbody>
</table>

Legend as in Table II

### References

42. JD Robertson (2012) Determination of Trace elements Levels in Human Plasma and Radiated Mice Tongue by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS).Missouri Univ.
44. GL Ellman (1959) Arch Bio chem Bio phys.82, 70.
46. SM Savita, SK Anitha, SD Chaya, a Bharati (2009) Hum Fertil.12, 28.
50. HS Lucky, G Sayal, K Yesul (2010) Female Infertility and Antioxidants. Curr Women's Heal Rev.6 84
58. IR Sweet, M Gilbert, E Maloney, DM Hockenbery, MW Schwartz, F Kim (2009) Diabetologia.52 921
64. VM Victor, M Rocha, C Banuls, A Alvarez, C de Pablo, M Sanchez-Serrano, M Gomez (2011) J Clin Endocrinol Metab.96 3115
68. M Li , Y Tang, C Li, Q Huang, D Le (2016) Biol Trace Elem Res. 176, 73.
70. Mishra R, Baveja V, Gupta (2013) Maedica (Buchar) 8, 328.
77. DD Watts (2003) Trace Elements and Other Essential Nutrients.4th Writ B-L-O-C-K Ed USA.