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Study of Trace Elements Selenium, Copper, Zinc and Manganese Level in Polycystic Ovary Syndrome (PCOS)

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ABSTRACT

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in premenopausal women, trace elements play an important role in PCOS, selenium performs various biological functions such as defense against oxidative stress, immune function and thyroid function, polycystic ovary syndrome (PCOS) is a common endocrine disorder in premenopausal women, trace Elements have important role in PCOS, selenium is involved in many biological functions, such as, protection against oxidative stress, immune function and thyroid function, Copper, zinc and manganese are essential micronutrients that have been integrated into various proteins and metalloenzymes and are active in the metabolic process of cells and in oxidative stress pathways that can lead to oxidative stress.

One hundred and twenty-four of patients' women with poly cystic ovarian syndrome (PCOS) patients and 56 normal ovulatory women participated in the study. Seleniumand serum Copper, zinc and manganese were measured by using flame atomic absorption spectrometry (FAAS).

Keywords-- Selenium, Copper, Zinc and Manganese, PCOS, Immune Function and Thyroid Function.

I. INTRODUCTION

The most common endocrine condition in women of reproductive age is polycystic ovary syndrome (PCOS). It was first identified in a published case series of seven patients with amenorrhoea and bilateral polycystic ovaries by Stein and Leventhal in 1935, and PCOS was originally named Stein-Leventhal syndrome1 in recent years., several terms, including polycystic ovarian syndrome (PCOS), follicular ovarian disease, O syndrome, functional ovarian hyperandrogenism, and ovarian dysmetabolic syndrome, have been used to describe this disorder. In actuality, polycystic ovaries are not the primary cause of amenorrhea or hirsutism in this condition. Rather, they are simply one sign of an underlying endocrinologic disorder that ultimately results in an ovulation (www.endocrineonline.org). In comparison to the normal ovary, the polycystic ovary has multiple small cysts .These cysts appear when frequent disruptions in menstrual cycles occur. The ovary is enlarged and produces excessive amounts of androgen and estrogenic hormones. This along with the absence of ovulation may cause infertility[2].

The common symptoms of Poly cystic ovarian syndrome[3] include Menstrual disorders: mostly oligomenorrhea or amenorrhea[4], Infertility, Multiple hormone imbalances, commonly include: androgens (testosterone), cortisol, estrogens, FSH (follicle stimulating hormone), insulin, LH (luteinizing hormone), progesterone, prolactin and thyroid hormones, Excessive production of masculine hormones, mostly acne and hirsutism, Metabolic syndrome as Metabolic diseases including insulin resistance, impaired glucose metabolism and dyslipidemia[(5-7)]

The exact cause of PCOS is still unknown, but Trace Elements have important role in PCOS and there are Some studies support this[8],Many trace elements are important for optimum human metabolic function. These micronutrients serve a variety of functions including catalytic, structural and regulatory activities in which, they interact with macromolecules such as enzymes, prohormones, and biological membrane receptors[9]. Others play a crucial role in the immune system Trace elements are uniquely required for maintenance of life and health. Lack or inadequate supply of such nutrients produces a functional impairment or can result in disease[10]. Numerous studies had revealed the role of increment of oxidative stress which could result from excessive production of reactive oxygen species in pathogenesis of polycystic ovary[11]. Over production of responsive oxygen species (ROS) is a typical component in women with polycystic ovary[12].

Selenium is involved in many biological functions, such as, protection against oxidative stress, immune function and thyroid function[13, 14]. Selenium is most widely recognized as a substance that speed up the metabolism of fatty acids and works together with vitamin E (Tocopherol) as antioxidant. Selenium also appears to work as an anti-inflammatory agent in certain disorders [15, 16].

Copper, zinc and manganese are fundamental micronutrients which joined into numerous proteins and metalloenzymes and they are dependable in cell metabolic system and oxidative stress pathways which may contribute to oxidative stress[17] Cu catalyzes the

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synthesis of highly reactive oxygen species (ROS) that causes oxidative damage to proteins, lipids, DNA and other molecules, Copper has an antioxidant action that protect cells from damage and is also a component of many enzymes that is responsible for the release of energy from carbohydrates, fat and protein. Copper is also important for formation of red blood cells, bone and connective tissues. PCOS may result in dysregulation of systemic copper homeostasis[18]. Another element of biological effect is magnesium and is essential to good health. As the second most abundant intracellular cation in the human body, magnesium is involved in more than 300 ATP and kinase-dependent enzymatic reactions[19]. Magnesium plays an essential physiological role in many functions of the body. It may play a role in glucose homeostasis, insulin action in peripheral tissues, and pancreatic insulin secretion, magnesium functions as a cofactor for several enzymes critical for glucose metabolism utilizing high energy phosphate bonds[20].

Zinc is directly involved in the synthesis, storage and secretion of insulin, as well as conformational integrity of insulintaste acuity and enhances the in vitro effectiveness of insulin. The function of zinc in the body metabolism is based on its enzymatic affinity and way of a zinc-enzyme complex or metallo-enzyme. Zinc is required for insulin synthesis and storage and insulin is secreted as zinc crystals. It maintains the structural integrity of insulin[21]. Zinc deficiency may play a role in the pathogenesis of polycystic ovarian syndrome and may be related with its long-term metabolic complications[22].

II. MATERIALS AND METHODS

The study was conducted in the Chemistry Department, College of Science, Basra University Samples were collected from "infertility center" at Basra hospital for Obstetrics and children and Faiha Hospital in Basrah Governorate-Iraq during the period from December 2017 till end of January 2019. The study was under taken to determine Trace Elements (Zn, Mg, Se, and Cu) changes in polycystic ovarian syndrome patients. The patients are already diagnosed as unexplained infertile women according to the basis of American Academy of Family Physicians[23]. In this study, 284 women volunteers were participated in the present study. One hundred and twenty-four of patients' women with poly cystic ovarian syndrome (PCOS) patients (60 patients with primary, 28 obese and 32 non-obese) and (64 patients with secondary, 31 obese and 33 non-obese) and 56 normal ovulatory women (27 obese and 29 non-obese) were followed up for 14 months, till end the study. While, 104 of women volunteers (70 patients and 34 healthy controls) were excluded from the study due to enable to follow up study. All the participants were of Basra governorate (Souther of Iraq). All of them were married women. The participants were in the age group of 18–45 years for patients and of 20–45 years for healthy control. Written informed consent was obtained from each participant. Women were in good health condition, without any other serious disorder. Exclusion factors were diseases affecting the metabolic state or not suitable to participate in this study. Women presenting endometriosis, uterine fibroid, breast cancer, epilepsy or migraine and those with hormone-dependent cancer were excluded. Furthermore, hype- and hypo-thyroidism, diabetes, mental disease, serious disease with dysfunction of heart, liver, kidney, were excluded as well as that using estrogen replacement therapy. The control group had regular menstrual cycles (26–30 day) and not used oral contraceptives for at least the preceding 3 months and had no clinical signs of hyperandrogenemia or any sign of PCOS symptoms.

Venous fasting blood samples (10 ml) were collected from patients and healthy volunteers by vein puncture then divided into two parts, the first (1 ml) was added into EDTA containing polypropylene tubes and shacked gently to be used for the measurement of the concentration of Se. The reminder was transferred to plain tube (without anticoagulant) which allowed clotting in a clean plain tube for 30 minutes at room temperature. After the blood had clotted it was placed in a centrifuge and spun at 402 x g for 10 minutes to obtain the serum. The obtained serum immediately use in detection of variables in this study, and others were stored in deep freezing at (-20ºC) until using.

2.1 Digestion Procedure of Selenium:

The blood must be digested in order to break out the organic materials associated with the selenium, so the samples were digested by transferring (1ml) of the whole blood into a Pyrex test tube, adding (1ml)of Conc. HNO_3 and (1ml) of Conc. H_2SO_4, placing in an oil bath at (130oC) until nitric acid (brown fumes) boiling away. Then, the tubes were removed from the oil bath and cooled to room temperature, followed by the addition of (1 ml) of 5 M $HClO_4$. They were placed again in an oil bath at (130oC) for 45 min. The tubes were removed again from oil bath and cooled to room temperature, then (1 ml) of 6 M HCl was added and the tubes were placed again in oil bath at (95oC) for 30 min then cooled to room temperature and diluted to (10 ml) by 6 M HCl[24].

2.2 Digestion Procedure of Zinc, Magnesium and Copper:

The samples were digested by adding (2 ml) of Conc. HNO3 and (1 ml) of Conc. HClO4 to (0.5 ml) serum in Pyrex tube, heated for (1 h) at (160oC) by oil bath. Until the digest becomes clear, then the tubes were cooled at room temperature, then the volume was completed to (10 ml) by 0.3 M HCl[25, 26]

2.3 Standard Solutions of Selenium (1000 ng/ml)

Stock standard solutions of selenium were prepared by dissolving (1.405 g) of selenium dioxide (SeO_2) in (5 ml) of HNO_3 by warming and diluting to exactly (1000 ml) with distilled deionized water. Then take (1ml) of stock solution and diluting to (100 ml) by 1.5 M HCl, (10 μg/ml) standard was formed. Further dilution by adding 1.5 M HCl to prepare standard

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calibration curve (5–25) ng/ml [27]

2.4 Standard Solutions of Zinc (1000 µg /ml)

Dissolving (0.1g) of zinc metal in (0.5 ml) of Conc. HNO_3, then complete the volume to 100 ml by distilled deionized water to prepare standard solution (1000 µg/ml). After that taking (1ml) of standard solution and diluting it to (100 ml) by 6 M HCl, a (10 µg/ml) standard was formed then it was used to make up (0.1–1.6)µg/ml stock standards. All stock standards were prepared by using deionized water as diluent[27].

2.5 Standard Solutions of Magnesium (1000 µg /ml)

Standard solutions of magnesium were prepared by dissolving (1.014gm) of $MgSO_4.7H_2O$ in water containing (0.5ml) of Conc. $HNO_3$and diluting the solution with distilled deionized water to (100 ml). This solution then was used to make up (0.02-2.5) µg/ml stock standards. All stock standards were prepared using deionized water as diluents[27].

2.6 Standard Solutions of Copper (1000 µg /ml)

(3.93gm) of $CuSO_4$. $5H_2O$ dissolving in water containing (1ml) of Conc. H_2SO_4 and diluting the solution with distilled deionized water to (100ml) to prepared standard solution of Cu .Then prepare standard solution at different Conc. (0.2-2)µg/ml[27]

2.7 Determination of Metals

Table (2.1) shows the optimum conditions for the determination of Se concentration used in this study according to previous studies (Al-AtbeeAA, 2013), and Table (2.2) shows the optimum conditions for the determination of Mg, Zn , Cu, The concentrations of Mg, Zn, Cu in standards and samples solutions were measured by flame atomic absorption spectrometry (FAAS).

Table (2.1): Optimized Conditions of Selenium Determination

Parameter	Optimized Condition
Flow Rate of Argon Gas	0.3 litter/min
Time of Mixing	(20-30) sec
HCl concentration	1.5 M
NaBH4 Concentration	2% w/v
NaBH4 Volume	200 µl
Se standard solution volume	200 µl
Lamp Current	10 mA
Wave length	196nm

Table (2.2): Optimized conditions of Mg, Zn Determination

Mattel / Parameter	Zn	Mg	Cu
Lamp Current (mA)	5	3	3
Wavelength (nm)	213.9	285.2	324.7
Slit Width	0.5	0.5	0.5
Slit Height	Normal	Normal	Normal
Read Time (s)	3	3	3

2.8 Statistical Analysis

The data were analyzed using SPSS software (Version 24), and thevalues were expressed as the mean values ± SD. P- values <0.05, 0.01were considered to be statistically significant, SD: Standard deviation, SE: Standard Errors, Range: is the difference between the highest and lowest values in the set, C.L: Confidence limits (Lower and Upper), P- value: N.S (P > 0.05), S (P < 0.05), HS (P < 0.01) indicate the level ofsignificance.

III. RESULTS

One hundred and twenty-four of patients' women with poly cystic ovarian syndrome (PCOS) were enrolled in this study. The patients women were distributed depending on the type of PCOS into two groups; 60 patients with primary (1ºPCOS, 28 obese and 32 non-obese) and 64 patients with secondary (2ºPCOS, 31 obese and 33 non-obese). All groups of PCOS women were compared with group of apparently healthy fertile control (fifty six) without significant difference in Age.

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			Se(ng/mL)	Mg(µg/mL)	Cu(µg/mL)	Zn(µg/Ml)
Mean± SD		PCOS1	42.6765±1.02	13.0818 ± 1.39	0.3415± 0.44	0.6346 ± 0.62
		PCOS2	44.0257± 0.59	13.1811 ± 1.08	0.3525 ± 0.13	0.63025 ± 0.71
SE		PCOS1	0.9622	0.7812	0.0432	0.0551
		PCOS2	1.3871	0.8677	0.1773	0.2265
Rang		PCOS1	33.23-51.97	5.73-19.12	0.301-0.378	0.616-0.654
		PCOS2	33.91-52.73	5.26-19.97	0.320 - 0.386	0.604 -0.657
95% C.I.	Upper	PCOS1	45.3675	15.4216	0.3817	0.6935
		PCOS2	46.2800	14.6883	0.3973	0.6889
	Lower	PCOS1	40.1855	11.0223	0.3062	0.5664
		PCOS2	41.7714	11.9838	0.3157	0.5775
Control Mean ± SD			55.2764± 0.44	15.5811± 1.88	0.23365 ± 0.23	0.7465 ± 0.41
p-value			H.S	H.S	H.S	H.S

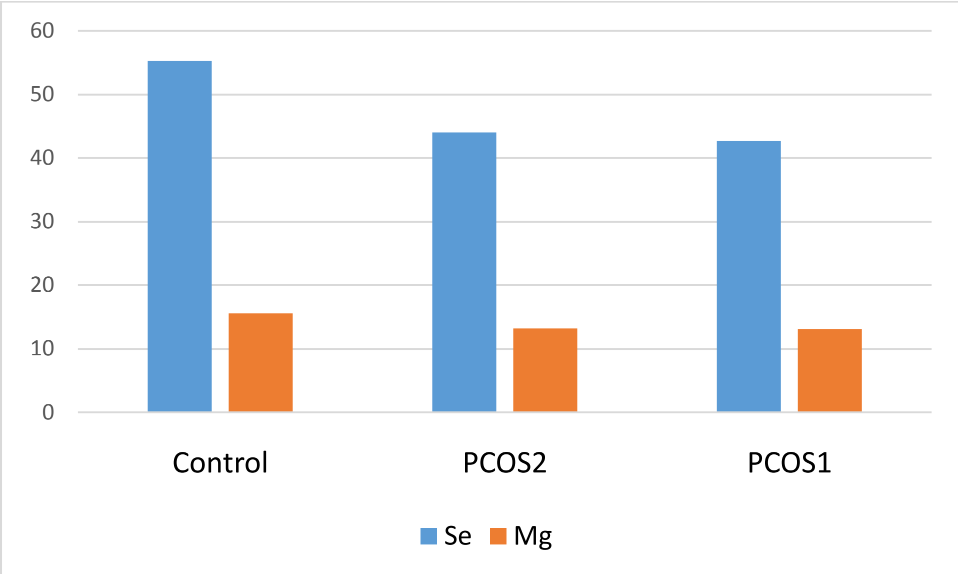

Figure 1: Level of (Se& Mg) in PCOS Patients and Control Group

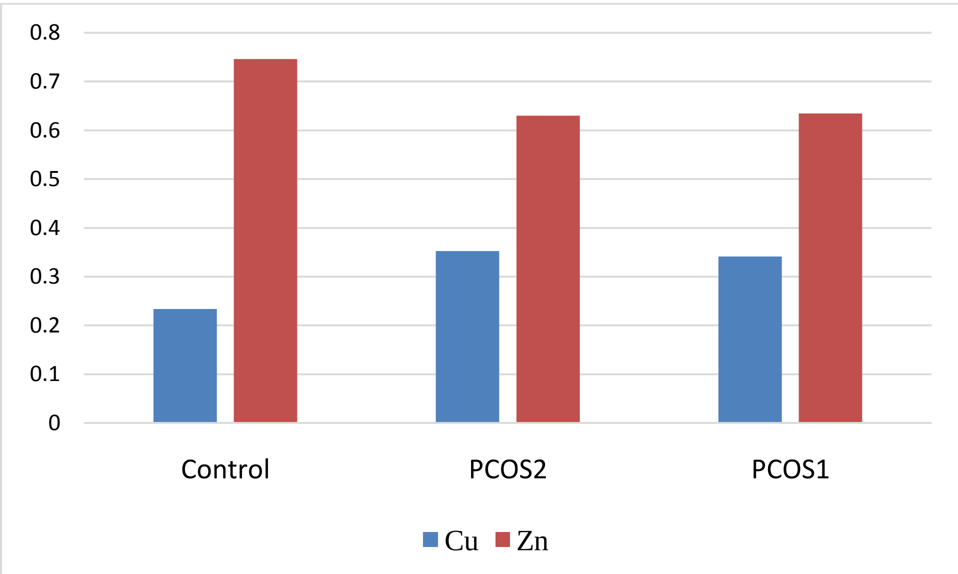

Figure 2: Level of (Cu&Zn) in PCOS Patients and Control Group

V. DISCUSSION

PCOS is a common female endocrinedisorder; Women with PCOS are known to be at increased risk of insulin resistance. There is a risk factor for developing type 2 diabetes mellitus, in these women. Adiposity plays a crucial role in the development and maintenance of PCOS and strongly influences the severity of both its clinical & endocrine features in many women with this condition.

In this study Se, Mg, Zn, Cu were analysed and compared with control groups to know the values of these parameters in PCOS

In this work, a significant decrease ($P < 0.01$) was seen in Selenium (Se) level in primary and secondary (obese- non obase), compared with that of control .On the other hand, there was no significant ($P>0.05$) when comparing the level of Se between primary and secondary (in both obese and non-obese PCOS patients), Decreased levels of whole blood Se in women with PCOS patients may be due to expose the subject to oxidative stress which is known to be associated with the pathogenesis of all known diseases [28]. The reduced level of Se in blood may be give an indication of increased the production of free radical and highly scavenging activity of either selenium or glutathione peroxidase enzyme. Also, there are some scientific reports have demonstrated that selenium plays a significant vital role in the undisturbed functioning of the reproductive system which prove to the correlations between the Se intake and the fertility as well as disorders of procreation processes[29]

In the current study, the Level of Mg was found to be significantly decreased ($P < 0.01$) in primary and secondary PCOS (obese-non obase) compared with that of control. Similar results were observed[8].On the other hand, there was no significant ($P>0.05$) when comparing the level of Mg between primary and secondary (in both obese and non-obese PCOS patients), Decreased levels of trace elements such as Mg have been shown in various reproductive events like infertility, spontaneous abortions, congenital anomalies, preeclampsia, placental abruption, premature rupture of membranes, still births and low birth weight [30].Furthemore, decreased level of Mg can paradoxically increased the risk factor of, or protect against oncogenesis. It has been reported that Mg is central in the cell cycle, and that its deficiency is an important conditioner in precancerous cell transformation. On the other hand, at increased levels, magnesium can cause damage to the brain, liver, kidneys, and the developing foetus[31]

In the current study, there were a significantly ($P < 0.01$) increased in level of Cu and significantly ($P < 0.01$) decreased in level of Zn in obese and non obese PCOS patients with primary and secondary, with compared to control. On the other hand, there was no significant change ($P>0.05$) when comparing the level of Cu and Zn between primary and secondary (in both obese and non-obese PCOS patients).

Both the trace elements Zn and Cu have a vital and necessary role as stabilizers, cofactors in many enzymes and essential elements for proper and best hormonal functions. In the state of Physio-chemically, the copper is considered as prooxidant and oxidant metal, while the Zn is able to acts as anti-oxidant by protecting the sulfhydryl groups of different proteins and enzymes against free radicals and inhibited the oxidative stress [32] .

Zinc is one of a trace elements crucial for normal insulin hormone response especially in the downstream insulin signalling and it is essential for normal insulin hormone synthesis, storage and release as well as it may be has an insulin-like activity upon binding to insulin receptor [33],On the other hand, the higher levels of Cu concentration in serum of women with PCOS may directly affect infertility rates by lowering progesterone levels resulting in annovulation, implantation failure or luteal phase deficit. Furthermore, in this work, it is uncertain whether high levels of copper are related to hidden inflammatory conditions or not. Also, the ceruloplasmin which is considered as a protein of the acute phase may be has ability to an increase in the serum copper and decrease in zinc levels [34]This may give an indication that imbalance in serum copper and zinc could be the major cause of infertility, and elevated level of this element may interfere with fertility in many possible ways; first, excessive amounts of copper may interfere with neuronal signaling in central nerve system (CNS) which are good responsible for the neuroendocrine regulation of fertility [35] .

VI. CONCLUSION

The results of this study may give an indication about the involvement of essential trace elements such as Se, Zn and Mg deficiency and/or copper overload as an important etiological role in the pathogenicity and increased complication of PCOS and therapeutic intervention with these trace elements as supplement in a suitable formula may be beneficial and might give a promise in this respect.

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