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Assessment of Some Trace Elements in Obese and Non-Obese Polycystic Ovary Syndrome (PCOS)

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Abstract: ***Objective: This research was designed to evaluate the serum levels of Copper, Zinc, Nickle, and Chromium in women with polycystic ovarian syndrome and to study its possible association with insulin resistance. Methods: Fifty-four women with PCOS and twenty-eight apparently healthy control women with regular menstruation with matching the age and BMI of the patient groups. There are twenty seven obese PCOS patients with BMI> 30 kg/m2 and another twenty-seven non-obese patient PCOS with BMI <30 kg/m^2. Controls divided into fourteen obese with BMI> 30kg/m^2 and fourteen non-obese with BMI <30 kg/m^2.Venous blood samples were collected to estimate serum levels of Copper, Zinc, Nickle, and Chromium. Results: The serum concentration of Copper (Cu) and Nickle (Ni) were significantly higher in PCOS Patients groups than controls groups, while the serum concentration of Zinc (Zn) was lowered in both obese and non-obese PCOS patients as compared to obese and non-obese controls samples respectively. There was no significant difference between obese PCOS women and non-obese PCOS women in the level of these elements. Conclusion Higher Serum Copper and Nickle with lower Zinc levels in PCOS patients than control subjects irrespective to BMI.***

Keywords: PCOS, Copper, Zinc, Insulin resistance, Oxidative stress

1. Introduction

The polycystic ovarian disorder is more widely recognized an endocrine problem in women, influencing 5% to 10% of women of reproductive age. (1) The main features of PCOS are insulin resistance anovulation and hyperandrogenism. Anovulation results in irregular menstruation, amenorrhea, ovulation-related infertility and polycystic ovaries. Hyperandrogenism results in acne and hirsutism. Insulin resistance is often associated with obesity, Type 2 diabetes, and high cholesterol levels. The symptoms and severity of the syndrome vary greatly among the affected women. Moreover, it may affect daily physical activities (2).

Insulin resistance is present in 65-80% of PCOS patients, causes early onset hyperglycemia and progression to type II diabetes, and also increases the risk of cardiovascular disease (3,4).IR occurs in both lean and obese women with PCOS. In contrast, in women without PCOS, insulin resistance occurs primarily in the obese. IR is an intrinsic part of the disease. Some believe that insulin resistance may be present in all women with PCOS. However, there is a lack of consistency in measuring for IR, and so some women remain undiagnosed (5).

The trace elements had been identified for an extended period as having the potential for intensifying metabolic diseases, e.g., pre-diabetes (metabolic disorder, insulin resistance, and obesity) or diabetes mellitus. Identifying the targets of cells and sites of activity of trace minerals had organized interest for their therapeutic uses. The activation signals of insulin receptor (chromium), antioxidant features (zinc, Copper has pro-oxidant and antioxidant properties (6), and Nickel causes an increased level of endogenous cellular hydrogen peroxide and its short lived reactive oxygen species. Nuclear protein damage caused by nickel reduces the enzyme activity needed for DNA replication, transcription, recombination, and repair. (7).

2. Aim of Study

To evaluate the serum levels of Copper, Zinc, Nickle, and chromium in infertile women with polycystic ovarian syndrome and to study its possible association with insulin resistance.

3. Literature Survey

This study was carried out at the Kamal Al-Samarrai Hospital (Center for Infertility treatment and in Vitro Fertilization "IVF"), and poisoning consultation center-Baghdad - Iraq from October /2016 to April/2017.

4. Subjects and Methods

Study population

The study included 82 volunteers of women, 54 of them were diagnosed patients with PCOS aged (18-39) years with mean age (obese 29.04± 5.64, non-obese 26.56±4.97) and 28 apparently healthy women were selected as controls, their age range was with (19-40) years with mean age (obese 32.07±5.86 and non-obese 24.64±4.25). Control groups have regular menstruation with matching the age and BMI of the patient groups. Every participant woman was questioned and asked to answer a specially designed interviewing format including; socio-demographic data, menstrual, obstetric, medical and family histories.

The diagnosis of PCOS in our study is based on the revised Rotterdam criteria, which require, two of the following three manifestations: (1) clinical and/or biochemical hyperandrogenism, (2) oligo-and/or anovulation (cycle length >35 days) and (3) polycystic ovaries on ultrasound (PCO was defined as the appearance of more than 11 follicles in each ovary, each measuring 2-9 mm in diameter, and/or increased ovarian volume > 10 ml) (8).All the patients were chosen under the supervision of a specialist gynecologist.

The studied women were divided into:

a) All the 54 study subjects diagnosing with PCOS divided into two groups based on their BMI
   1) 27 obese PCOS patients with BMI> 30 kg/m^2 (mean 34.81±0.820)
   2) 27 non-obese patients PCOS with BMI <30 kg/m^2 (mean 26.41±0.401)
b) The 28 apparently healthy control women did not have symptoms of hyperandrogenism, a history of menstrual dysfunction, infertility, or sonographic signs of PCOS. There were divided into:
   1) 14 obese control subjects with BMI> 30kg/m^2(mean 32.81±0.720)
   2) 14 non-obese control subjects with BMI <30 kg/m^2. (mean 23.90±0.678)

5. Specimens Collection and Preparation

The venous blood samples (10 cc) was taken from each woman of PCOS and control group during the early follicular phase. Between days 2 and 4 of the spontaneous bleeding episode after overnight fasting 12 hours. Blood samples were put in gel tubes without anticoagulant then samples left for 30 minutes to clot. After complete clotting, the serum is separated by centrifugation (centrifuged for 10 minutes at 3500 to 4000 rpm to obtain serum).The collected serum was divided into 10 Eppendorf tubes (9 of them kept frozen (-80˚C) until their assay and one Eppendorf used for direct measuring of fasting glucose level).

6. Assay

The serum levels of Copper, Zinc, Chromium, and Nickle were measured by atomic absorption spectrophotometer in poisoning consultation center in Iraq. The Atomic absorption spectrophotometric method is an optimal choice to measure the amount of trace elements and heavy metals in serum owing to its high sensitivity and specificity [(9)].

The serum Copper and serum Zinc concentrations were analyzed at the poisoning consultation center by flame atomic absorption spectrophotometer (analytic Jena, NovAA300, Germany and Buck ,210VGP, USA) following standardized procedure while the serum Chromium and serum Nickle concentrations were measured by flameless atomic absorption spectrophotometer (FAASP) measurements that are carried out on Buck 210VGP, USA. FAASP, it is an analytic method for determination of elements and is applicable for the analysis of concentrations ranging from a trace up to large concentration [(10)].

Serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and total testosterone (TES) were tested by automated quantitative test (ELFA) (VIDAS® PC Autoanalyzer, Biomerieux, Italia)[(11,12)]. Fasting serum glucose (FSG) was evaluated according to the method of Barham and Trindoer (1972) [(13)]. Fasting serum insulin and serum sex hormone binding globulin (SHBG)(Demeditec Diagnostics GmbH, Germany); were determined with enzyme-linked immunosorbent assay (ELISA) according to manufacturers' instructions [(14,15)].

The instrument used in this study show in the table 1.

Estimation of insulin resistance homeostatic model assessment– insulin resistance **(HOMA-IR)** was calculated from fasting glucose (FG) and fasting insulin (FI) using the following formula [(16)]:

HOMA-IR = {Fasting insulin (µIU/ml) x Fasting glucose (mmol/l)}/ 22.5

Free Androgen Index (FAI) is a ratio used to determine abnormal androgen status in individuals. The ratio is the total testosterone level divided by the sex hormone binding globulin (SHBG) level and then multiplying by a constant, usually 100 [(17)].

$$FAI = \frac{\text{Total testosterone } (\frac{\text{nmol}}{\text{l}})}{\text{SHBG } (\frac{\text{nmol}}{\text{l}})} \times 100$$

7. Results

The anthropometric and demographic characteristics of polycystic ovary syndrome (PCOS) patients and controls are summarized in Table 2.

As shown in Table 3, the serum FSH level was significantly lower in obese PCOS patients than control both obese and non-obese (p = 0.004, p= 0.030 respectively), also there was no significant difference between obese and non-obese PCOS at p>0.05 while the serum LH, LH/FSH ratio, and total testosterone levels were elevated in both obese and non-obese PCOS patients as compared to their corresponding controls, obese and non-obese respectively but there was no significant difference in serum levels of these hormones between PCOS patients groups as well as between controls groups.

The serum of SHBG in the obese PCOS was lower than the non-obese control samples, but there was no significant difference among the remaining studied samples. In the PCOS patients (both obese and non-obese) Free androgen index (FAI), which is calculated from total testosterone and SHBG, was significantly higher from their corresponding controls samples (p = 0.001, p = 0.039 respectively), but there was no significant difference between obese and non-obese PCOS (p =1.000) as well as between obese and non-obese control samples (p =0.772).

Serum glucose levels were significantly higher in obese PCOS patients when compared with obese and non-obese controls samples (p = 0.012, p = 0.002 respectively) but there was no significant difference between non-obese PCOS and their corresponding controls (p = 0.068).

Serum insulin levels and HOMA-IR were significantly higher in obese PCOS than the non-obese PCOS (P= 0.024, P= 0.015). Also, there were significantly elevated in obese PCOS patients as compared with the obese controls and non-obese controls, but there was no significant difference between controls groups.

Table 4 explained that serum copper (Cu) was significantly higher in PCOS Patients than controls, but there was no significant difference between obese PCOS and non-obese

PCOS. Furthermore, copper was significantly elevated in obese and non-obese PCOS (159.5± 2.94, 165.9±4.40 respectively) as compared to obese and non-obese controls samples (126.4±4.56, 111.9±4.43 respectively). The serum Nickle was higher in obese and non-obese PCOS as compared with non-obese control samples (obese PCOS $p<0.000$ and non-obese PCOS $p< 0.000$), but there was no significant difference between PCOS groups as well as between controls groups. While the serum Zinc (Zn) level was not significantly different between obese PCOS women and non-obese PCOS women (Zn) $p< 0.998$ as well as between obese and non-obese controlled samples ((Zn) $p<0.694$). But the serum concentrations of Zn were lowered in both obese and non-obese PCOS patients (72.52 ±2.33, 72.04 ± 1.514 respectively) as compared to obese and non-obese controls samples (109.8±5.76, 101.7±4.57 respectively). There was no significant difference between obese and non-obese PCOS patients also between controls groups in the serum chromium levels as shown in table 4.

8. Correlation Studies

Correlation values that presented in this section were calculated using Pearson s correlation coefficient, considering P values < 0.05 as the level of significance are summarized as follows:

1. Pearson's Correlation in Obese PCOS Women

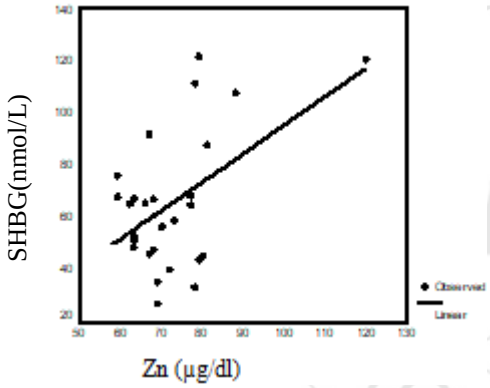

Figure 1: correlation between serum Zinc (Zn) level and serum of sex hormone binding globulin (SHBG) in obese PCOS patients (r = 0.502 p < 0.05).

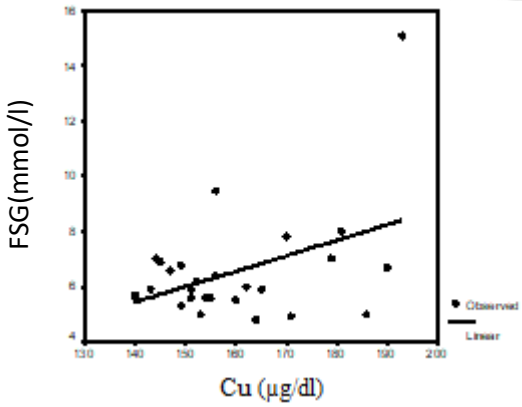

Figure 2: correlation between serum Copper (Cu) level and fasting serum glucose (FSG) in obese PCOS patients (r = 0.425 p < 0.05).

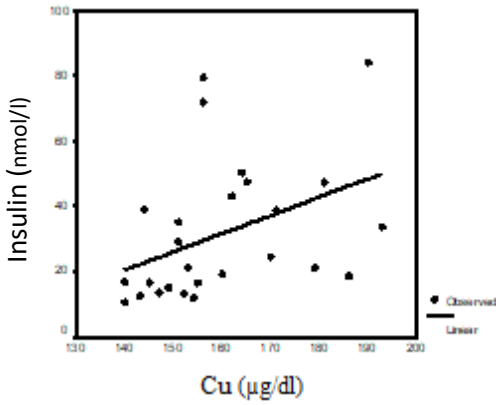

Figure 3: Correlation between serum Copper (Cu) level and fasting serum insulin in obese PCOS patients (r = 0.408 p < 0.05).

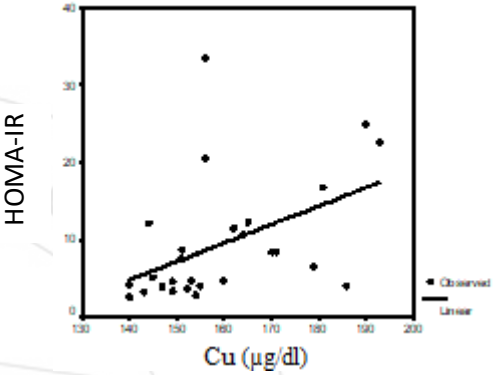

Figure 4: Correlation between serum Copper (Cu) level and HOMA-IR in obese PCOS patients (r = 0.464 p < 0.05).

2. Pearson's Correlation in Non- Obese PCOS Women

In our studies, there was no correlation between studied hormones and elements in non- obese PCOS women.

Table 1: Instrument and their supplier

Instruments	Supplier
a, NovAA300Analytik jen	Germany
Buck 210VGP,flameless	USA
Buck 210VGP,flame	USA
Cecil(CE 7200)Spectrophotometer	England
DI washer II (ELISA-Plate Washer)	AustriaDIALAB,
Dionizer, pur1TE	ONDEO, UK
ELISA plate reader	Beckman coulter, Austria
(test tube shaker)	TKA226, Italia
Microshaker Dynatech(ELISA-Plate shaker)	Biosan, UK
Magnetic stirrer , hotplate	Stuart scientific. UK
Micropipette 20-200µl	Humapette, Germany
Micropipette 10-1000µl	watson, Japan
Multichannel-Micropipette 10-1000 µL	Brand, Germany
Water path	Memmert, Germany
VIDAS® PC Autoanalyzer	Biomerieux, Italia

Table 2: The anthropometric and demographic Data of PCOS patients and controls

Variables \ Groups	PCOS		Controls		P-value
	obese(27)	non-obese(27)	obese(14)	non-obese(14)	
AGE	29.04 ± 1.09	26.56 ± 0.96	32.07 ± 1.57	24.64 ± 1.14	0.204
BMI(kg/m^2)	34.81 ± 0.82	26.41 ± 0.40	32.81 ± 0.72	23.90 ± 0.67	0.000**
Waist (cm)	103.48±2.81	87.76±1.68	100.00±1.96	76.64±2.04	0.000**
Hip(cm)	116.9 ± 2.73	103.6 ± 1.64	119.4 ± 1.58	94.00 ± 0.70	0.000**
WHR	0.885±0.011	0.847±0.012	0.838±0.012	0.782±0.020	0.000**
F.Hx of PCOS	9 (33.3%)	5 (18.5%)	0	0	0.214
F.Hx of obesity	13 (48.1%)	2 (7.4%)	2(14.2%)	0	0.001**
U/S for PCOS	+	+	-	-	-
A.N.	8 (29.6%)	4 (14.8%)	0	0	0.190
Hirsutism	24(88.9%)	24(88.9%)	0	0	1.000
Acne	8(29.6%)	10(37%)	0	0	0.564
Male pattern baldness	10(37%)	9(33.3%)	0	0	0.776
OM	20(74.1%)	17(63%)	0	0	0.379
AM	6(22.2%)	8(29.6%)	0	0	0.535
Regular cycle	1(3.7%)	2(7.4%)	14(100%)	14(100%)	1.000

*The data are expressed as the numbers (percentage) or mean ± standard error of the mean (SEM). **P < 0.01 are highly significantly different. F.Hx: family history, U/S: ultrasound, A.N: acanthosis nigricans, OM: Oligomenorrhea, AM: amenorrhea.*

Table 3: Descriptive Statistics of studied hormones with Comparative significant studies between groups (A) and groups (B)in serum levels of this hormones

Variables	**Groups**	Mean ±SE	(A) Groups	(B) Groups	Pvalue*
FSH	Obese PCOS	4.592 ± 0.194	Obese PCOS	Obese Control	0.004 (HS)
				Non Obese PCOS	0.348(NS)
	Obese control	6.499 ± 0.790		Non Obese Control	0.030 (S)
	Non-obese PCOS	5.092 ± 0.332	Obese Control	Non Obese Morbid	0.031(S)
				Non Obese Control	1.508 (NS)
	Non-obese Control	6.010 ± 0.657	Non Obese PCOS	Non Obese Control	0.156 (HS)
LH	Obese PCOS	5.006 ± 0.389	Obese PCOS	Obese Control	0.001 (HS)
	Obese control	2.840 ± 0.315		Non Obese PCOS	1.000 (NS)
				Non Obese Control	0.000 (HS)
	Non-obese PCOS	5.000 ± 0.428	Obese Control	Non Obese PCOS	0.001(HS)
				Non Obese Control	0.882 (NS)
	Non-obese Control	2.541 ± 0.259	Non Obese PCOS	Non Obese Control	0.000 (HS)
LH/FSH Ratio	Obese PCOS	1.152 ± 0.114	Obese PCOS	Obese Control	0.000 (HS)
				Non Obese PCOS	0.985 (NS)
	Obese control	0.448 ± 0.068		Non Obese Control	0.000 (HS)
	Non-obese PCOS	1.095 ± 0.112	Obese Control	Non Obese PCOS	0.000 (HS)
				Non Obese Control	0.993 (NS)
	Non-obese Control	0.465 ± 0.055	Non Obese PCOS	Non Obese Control	0.000 (HS)
TES	Obese PCOS	3.078 ± 0.363	Obese PCOS	Obese Control	0.000 (HS)
				Non Obese PCOS	0.882(NS)
	Obese control	1.229 ± 0.104		Non Obese Control	0.000 (HS)
	Non-obese PCOS	2.667 ± 0.424	Obese Control	Non Obese PCOS	0.013 (S)
				Non Obese Control	0.992 (NS)
	Non-obese Control	1.175 ± 0.163	Non Obese PCOS	Non Obese Control	0.012 (S)
SHBG	Obese PCOS	65.69 ± 5.04	Obese PCOS	Obese Control	0.123(NS)
				Non Obese PCOS	0.154(NS)
	Obese control	86.88 ± 12.62		Non Obese Control	0.035(HS)
	Non-obese PCOS	81.88 ± 9.98	Obese Control	Non Obese PCOS	0.714 (NS)
				Non Obese Control	0.611 (NS)
	Non-obese Control	94.85± 9.42	Non Obese PCOS	Non Obese Control	0.343(NS)
FAI	Obese PCOS	5.221 ± 0.669	Obese PCOS	Obese Control	0.001 (HS)
				Non Obese PCOS	1.000(NS)
	Obese control	1.998 ± 0.429		Non Obese Control	0.000 (HS)
	Non-obese PCOS	5.076 ± 1.228	Obese Control	Non Obese PCOS	0.104 (NS)
				Non Obese Control	0.772 (NS)
	Non-obese Control	1.500± 0.289	Non Obese PCOS	Non Obese Control	0.039 (S)
FSG	Obese PCOS	6.530 ± 0.387	Obese PCOS	Obese Control	0.012 (S)
				Non Obese PCOS	0.122(NS)
	Obese control	5.229 ± 0.149		Non Obese Control	0.002 (HS)

	Non-obese PCOS	5.874 ± 0.312	Obese Control	Non Obese PCOS	0.207 (NS)
				Non Obese Control	0.616 (NS)
	Non-obese Control	4.936± 0.193	Non Obese PCOS	Non Obese Control	0.068 (NS)
Insulin	Obese PCOS	31.34 ± 4.04	Obese PCOS	Obese Control	0.002 (HS)
				Non Obese PCOS	0.024(S)
	Obese control	13.75± 1.60		Non Obese Control	0.009 (HS)
	Non-obese PCOS	18.21 ± 1.63	Obese Control	Non Obese PCOS	0.223 (NS)
				Non Obese Control	0.998 (NS)
	Non-obese Control	14.36± 3.03	Non Obese PCOS	Non Obese Control	0.683 (NS)
HOMA-IR	Obese PCOS	9.513 ± 1.512	Obese PCOS	Obese Control	0.002 (HS)
				Non Obese PCOS	0..015(S)
	Obese control	3.142 ± 0.340		Non Obese Control	0.002 (HS)
	Non-obese PCOS	4.500 ± 0.329	Obese Control	Non Obese PCOS	0.034 (S)
				Non Obese Control	0.999 (NS)
	Non-obese Control	3.058± 0.590	Non-Obese PCOS	Non-Obese Control	0.175 (NS)

(*) HS: Highly Significant. at P<0.01; S: Significant. at P<0.05; NS: Non Significant at P> 0.05

Table 4: Descriptive Statistics of trace elements with comparative significant studies between groups (A) and groups (B)in blood levels of studied elements

Variables	**Groups Numbers**	Mean ±SE	(A) Groups	(B) Groups	P-value*
Zinc (Zn)	Obese PCOS	72.52 ± 2.33	Obese PCOS	Obese Control	0.000 (HS)
	Obese control	109.8 ± 5.76		Non Obese PCOS	0.998(NS)
	Non-obese PCOS	72.04 ± 1.514		Non Obese Control	0.000 (HS)
	Non-obese Control	101.7 ± 4.57	Obese Control	Non Obese PCOS	0.000 (HS)
				Non Obese Control	0.694 (NS)
			Non Obese PCOS	Non Obese Control	0.000 (HS)
Copper (Cu)	Obese PCOS	159.5 ± 2.94	Obese PCOS	Obese Control	0.000 (HS)
	Obese control	126.4 ± 4.56		Non Obese PCOS	0.210(NS)
	Non-obese PCOS	165.9 ± 4.40		Non Obese Control	0.000 (HS)
	Non-obese Control	111.9 ± 4.43	Obese Control	Non Obese PCOS	0.000 (HS)
				Non Obese Control	0.043 (S)
			Non Obese PCOS	Non Obese Control	0.000 (HS)
Nickle (Ni)	Obese PCOS	0.020 ± 0.001	Obese PCOS	Obese Control	0.752 (NS)
	Obese control	0.013 ± 0.003		Non Obese PCOS	0.649(NS)
	Non-obese PCOS	0.018 ± 0.001		Non Obese Control	0.000 (HS)
	Non-obese Control	0.010 ± 0.001	Obese Control	Non Obese PCOS	0.752 (NS)
				Non Obese Control	0.750 (NS)
			Non Obese PCOS	Non Obese Control	0.000 (HS)
Chromium (Cr)	Obese PCOS	0.242 ± 0.055	Obese PCOS	Obese Control	0.352(NS)
	Obese control	0.149 ± 0.007		Non Obese PCOS	0.998(NS)
	Non-obese PCOS	0.230 ± 0.052		Non Obese Control	0.435 (NS)
	Non-obese Control	0.158 ± 0.006	Obese Control	Non Obese PCOS	0.426 (NS)
				Non Obese Control	0.787 (NS)
			Non-Obese PCOS	Non-Obese Control	0.522 (NS)

(*) HS: Highly Significant. at P<0.01; S: Significant. at P<0.05; NS: Non Significant at P> 0.05

9. Discussion

The diagnosis of PCOS in this study depends on Rotterdam criteria that are confirmed by the results obtained from analysis data related to the measured hormones [18]. In the table (3) the serum FSH level was lowered in PCOS patients, in contrast, to control groups while, the higher level of LH in both obese and non-obese PCOS as compared to control obese and non-obese respectively. Hence the LH: FSH ratio was significantly elevated in PCOS patients in both obese & non-obese. The serum levels of (LH, FSH LH/FSH ratio, prolactin, total testosterone, free androgen index (FAI) and TSH) are not significantly different between obese and non-obese PCOS patients. Additionally, we found that the elevated level of LH relative to FSH, increase LH/FSH ratio and higher total testosterone in patient with PCOS especially in obese one as compare to control subjects, this result was agreeing with Neoklis AG et al. (2016) who found that the LH, LH/FSH ratio, total testosterone and FAI were elevated in women with PCOS as

compare to control and lower FSH level in PCOS women than control [19].

In our study, we found that significantly low level of SHBG in obese PCOS patients as compared with the non- obese control. In comparison with other studies as seen in the table(3), Cupisti et al. reported that obese PCOS women are mainly associated with significantly increased FAI, and decreased SHBG [20], an observation that was also approved by Meuller et al. [21]. Some research suggests that the FAI the best marker for predicting PCOS in women [22].

In tables (3) explained that the metabolic picture of PCOS was documented by determined the insulin level through the measured data related to insulin resistance which appears as elevated fasting serum glucose level in obese PCOS women when compared to control groups but there was no significant difference between obese PCOS and non-obese PCOS. Also, the obese PCOS has highest insulin level than the non-obese PCOS that indicating the obesity is an important role in insulin resistance which is documented by highest HOMA-IR in obese PCOS than the non-obese PCOS. This result was confirmed by Behboudi-Gandevani S et al., who found that higher serum insulin and HOMA-IR in obese PCOS than the non-obese PCOS [23].

A study by Stepto N. K., show that hyperinsulinemia is present in 85% of patients with PCOS, including 95% of obese and 65% of lean affected women, this study in agreement with our result which is higher fasting insulin level in obese PCOS women [24].

In this study, we found that serum adiponectin level significantly lowered in PCOS patients from their control, decrease the serum adiponectin level in obese PCOS when compared to that of corresponding controls subjects as shown in the tables (3). This result enforces other studies which documenting decreased serum levels of adiponectin in PCOS women compared with weight- and BMI-matched controls [25].

PCOS women show hypoadiponectinemia. Authors suggested that obesity, insulin resistance, or hyperandrogenemia may be the cause of hypoadiponectinemia in women with PCOS, the metabolic mechanism of adiponectin is not entirely understood, but regulation of glucose and lipid metabolism via stimulation of fatty acid oxidation, suppression of hepatic glucose output and increased insulin sensitivity in liver and skeletal muscles are identified to be key roles of adiponectin [26].Therefore, Decreased adiponectin plasma levels are associated with insulin resistance, obesity and type 2 diabetes mellitus [27].

There are several investigations suggested that metals might be involved in the development of PCOS. However, few studies have directly examined serum trace element concentrations about PCOS Keeping in view the significant lack of toxicity data for the effects of trace elements on PCOS [28], the present study was designed to investigate serum heavy metal and trace element concentrations about hormone levels and PCOS.

Tables 4 reveal that Statistically significant higher serum Copper (Cu) and serum Nickle (Ni) concentrations in the PCOS than controls with the lower serum concentration of Zinc (Zn) in PCOS groups as compared to their control groups. There was no significant difference among the all studied groups in serum level of Chromium (Cr). Furthermore, there was no significant difference between obese and non-obese PCOS patients in the serum concentration of measured trace element.

Tables 4 show that the results of serum zinc level lower in PCOS than control. This result in agreement with other studies which observed that serum zinc levels are significantly lower in PCOS patients than control subject [29,30]. The relationship between serum zinc level and pathogenesis of PCOS related to insulin resistance and oxidative stress due to the zinc deficiencies in PCOS patient lead to the development of insulin resistance and hyperinsulinemia which in turn progress to long-term risks of T2DM [31].

Several mechanisms have been suggested to explain the association between zinc and insulin resistance. Zinc plays a significant role in the stabilization of insulin hexamers and the pancreatic storage of insulin because it can improve insulin binding to hepatocyte membranes. In fact, reduced hepatic insulin binding to hepatocyte membranes during zinc deficiency may be associated with the contribution of zinc during insulin receptor synthesis [32].

Moreover, Zinc can activate the insulin signaling pathway through inhibition of protein tyrosine phosphatase leading to increased phosphorylation of the insulin receptor. Also, Zinc ions can work in an insulin mimetic way within adipocytes, stimulating lipogenesis and glucose transport through translocation of glucose transporter 4 (GLUT4) to the cell membrane [33].

The compensatory hyperinsulinemia occurring as a result of insulin resistance state lead to a decrease of SHBG, there is statistically significant correlation between serum zinc and serum SHBG as shown in the figure 1.

Other mechanisms of zinc's relationship to PCOS may be its effect on oxidative stress; zinc is co-factors of antioxidant enzymes such as catalase and SOD. Hyperinsulinemia and/or hyperglycemia also have the appositive impact on oxidative stress. It is possible that, in patients with PCOS, decreased antioxidant capacity can be aggravated by Zn deficiency and IR [34].

In our study, statistically significantly higher serum Copper concentrations in the PCOS group compared to the control group were observed, table 4.which was agreed with Kurdoglu Z, et al., who showed The PCOS patients had a statistically and significantly higher amount of Copper than in the control group [28].

A recent study found that fasting glucose increases with Copper levels in individuals with PCOS [29]. In our study there was a statistically significant correlation of serum copper with fasting glucose level, fasting serum insulin and HOMA-IR as showed in the figure 2, 3, 4; So the Copper has a role in exacerbating of insulin resistance in PCOS patients.

Other study showed that significantly higher values of serum Cu (P<0.04) are also found in insulin resistant women with PCOS compared to the controls [34]. The mechanisms of the effect cupper on PCOS other than insulin resistance is the generation of ROS, decreasing glutathione levels and serve as a cofactor for many enzymes involved in redox reactions, such as cytochrome c oxidase or superoxide dismutase. [35,36]. In PCOS, stimulation of ROS generation from mononuclear cells (MNCs) by hyperglycemia may play a role in inflammation through the release of tumor necrosis factor-α from circulating MNCs. Thus, ROS generation from MNCs in response to hyperglycemia may serve as an inflammatory trigger for the induction of IR in PCOS [37].

So the Copper has a significant role in the generation of insulin resistance through the mechanism related to oxidative stress by the formation of ROS.

High Copper concentrations can contribute to the release of LH and adrenocorticotropic hormone by affecting the pituitary gland, which affects ovulation. Other research has shown that copper and zinc may cause oxidative stress to act on PCOS by affecting the hormone levels [29]. In the current study, PCOS patients had higher levels of LH than patients without PCOS; this finding documented the previous study.

Chromium acts directly on insulin receptor mechanisms that augment cellular glucose uptake [38]. In our study serum, chromium level was not statistically differenced among the all studied groups of PCOS and controls; Zheng G et al. was confirmed this result [29].

Although, the serum Nickle (Ni) level within the normal range (0.010 – 0.030 µg/dl), there was a statistically significant difference in obese PCOS with non-obese control samples (p=0.000) and non-obese PCOS with corresponding groups (p= 0.000). We found the single work of Chinese investigators which revealed an increase in serum Nickel levels in PCOS women [293] which was confirmed by the results of our study.

It is known that Nickle can cause dysfunction of cellular membranes and mitochondria, DNA molecules rupture with damage of transcription and RNA synthesis, activate lipid peroxidation and to decrease antioxidant systems activity. For these reasons, accumulation of the excessive amount of nickel may participate in damaging of folliculogenesis and ovulation in PCOS patients.

It seems that Nickel disrupts physiological homeostasis, inducing glucose deregulation through ROS pathways [39] Indeed, previous studies have reported an association between increased Nickel concentration and an elevated prevalence of type 2 diabetes in humans [40,41].

10. Conclusion

A possible association is perceptible in our study between serum elements and women with PCOS. High serum copper and Nickle with low Zinc levels are associated with PCOS in our study. Chronic copper overload may have exaggerated insulin resistance that is associated with PCOS and deficiency of Zinc cause oxidative stress to act on PCOS by affecting the hormone levels; these results provide clues to explore the mechanism of PCOS and guidance for element treatments in PCOS patients in clinical trials related to antioxidant supplementation in PCOS. Further studies for evaluating the effect of copper and Zinc in PCOS patients related to oxidative stress and insulin resistance.

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