A comparative Study of Blood Levels of Manganese, Some Macroelements and Heavy Metals in Obese and Non-Obese Polycystic Ovary Syndrome Patients

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Abstract

Polycystic ovary syndrome (PCOS) is a prevalent condition in women of reproductive age. It is characterized by androgen excess and chronic anovulation. Some trace elements, macroelements, and heavy metals have been linked to pathophysiological mechanisms of PCOS .

To study the alterations in the serum levels of the trace element manganese (Mn), some macroelements, magnesium(Mg) and calcium (Ca), and the heavy metals cadmium (Cd) and lead (Pb), in obese and non-obese PCOS patients; and the association of these alterations with some of the hormonal changes occurring in PCOS.

The study was carried out at Kamal Al-Samarrai Hospital (Center for Infertility treatment and *in vitro* Fertilization "IVF") Baghdad- Iraq. Eighty-two women were enrolled in the study. Fifty-four of them were diagnosed by a specialist gynecologist as PCOS patients; they were subdivided into two subgroups according to their body mass index (BMI); twenty-seven obese PCOS patients with BMI > 30 kg/m^2, and another twenty seven non obese patients PCOS with BMI <30 kg/m^2. Whereas, twenty-eight apparently healthy women with regular menstruation and of comparable age, were selected to serve as control groups; they were subdivided into, fourteen obese women with BMI > 30kg/m^2, and fourteen non obese women with BMI <30 kg/m^2.

Blood lead and cadmium levels were significantly higher in both of the obese and the non-obese PCOS groups, than in their corresponding control groups. While, serum magnesium, calcium and manganese levels were significantly lower in both of the obese and the non-obese PCOS groups, as compared to their corresponding control groups. The results revealed no significant difference in the levels of the measured elements, between the obese PCOS group and the non-obese PCOS group. The serum FSH levels was significantly lower in obese PCOS patients than in the obese and non-obese control groups. There was a positive correlation between blood lead and serum TSH levels in non-obese PCOS women; and between serum total testosterone and cadmium levels in obese PCOS women. Finally, there was negative correlation between serum magnesium and serum LH levels in non-obese PCOS women.

the study has demonstrated higher blood levels of lead and cadmium; and lower serum levels of magnesium, calcium and manganese in PCOS groups than control subject. There were no significant differences between obese PCOS women and non-obese PCOS women in the levels of the studied hormones, elements and heavy metals.

Keywords: PCOS, Trace elements, Macroelements, Heavy metals, infertility.

دراسة مقارنة لمستويات الدم من المنغنيز وبعض العناصر الرئيسية والفلزات الثقيلة في البدينات وغير البدينات من المريضات المصابات بمتلازمة المبيض متعدد الاكياس

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الخلاصة

متلازمة المبيض متعدد الاكياس هي حالة سائدة لدى النساء في سن الإنجاب. وتتميز بزيادة الاندروجين وعدم الاباضة المزمن. ان بعض العناصر النزرة ، العناصر الرئيسية والفلزات الثقيلة قد ترتبط بالآليات المرضية الفيزيولوجية لمتلازمة المبيض متعدد الاكياس. دراسة ارتباط التغيرات الهرمونية مع التغيرات في مستوى المصل لعنصر المنغنيز ،بعض العناصر الرئيسية من المغنيسوم والكالسيوم ومستوى الدم الكلي من الفلزات الثقيلة الكادميوم والرصاص بين النساء البدينات وغير البدينات المصابات بمتلازمة المبيض متعدد الاكياس .أجريت الدراسة في مستشفى كمال السامرائي (مركز علاج العقم والتخصيب في المختبر) بغداد - العراق. شملت الدراسة ٨٢ متطوعة من النساء، تم اختيار ٥٤ منهن مريضات بمتلازمة المبيض متعدد الاكياس تحت إشراف أخصائية نسائية وتوليد وتم تقسيمهن إلى مجموعات فرعية وفقا لمؤشر كتلة الجسم الى: ٢٧ مريضة بمتلازمة المبيض متعدد الاكياس اللواتي يعانين من السمنة المفرطة و ٢٧اخريات لا يعانين من السمنة المفرطة في حين تم اختيار ٢٨ امرأة على ما يبدو بحالة صحية جيدة ، تم تقسيمهن إلى ١٤ يعانين من السمنة و١٤لا يعانين السمنة مع انتظام الدورة الشهرية. طيف الامتصاص الذري هو طريقة تستخدم لقياس كمية من العناصر النزرة والفلزات الثقيلة في كل العينات.

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اظهرت الدراسة ان الرصاص والكادميوم أعلى بكثير في مجموعة المريضات اللواتي يعانين من متلازمة المبيض متعدد الاكياس مقارنة بمجموعة النساء الطبيعيات، في حين انخفاض المغنيسيوم والكالسيوم والمنغنيز في مريضات بمتلازمة المبيض متعدد الاكياس البدينات وغير البدينات على حد سواء بالمقارنة مع النساء الطبيعيات البدينات وغير البدينات على التوالي. لم تكشف النتائج عن وجود فرق معنوي بين النساء اللواتي يعانين من متلازمة المبيض متعدد الاكياس البدينات وغير البدينات في مستوى العناصر المقاسة.

هناك علاقة طردية بين مستويات الدم من الرصاص مع مستويات المصل من الهرمون المحفز للغدة الدرقية في مجموعة المريضات اللواتي يعانين من متلازمة المبيض متعدد الاكياس غير البدينات بالا ضافة الى ذلك كان هناك ارتباط طردي بين مستويات الدم من الكادميوم ومستويات مصل الدم من التيستوستيرون في مجموعة المريضات اللواتي يعانين من متلازمة المبيض متعدد الاكياس البدينات. وعلاوة على ذلك، كان هناك ارتباط عكسي بين المغنيسيوم في الدم ومستويات المصل من هرمون LH في النساء اللواتي يعانين من متلازمة المبيض متعدد الاكياس غير الدينات

ارتفاع مستويات الدم من الرصاص والكادميوم في حين انخفاض مستويات المصل من المغنيسيوم والكالسيوم والمنغنيز في مجموعة المريضات اللواتي يعانين من متلازمة المبيض متعدد الاكياس مقارنة بمجموعة النساء الطبيعيات. لم تكن هناك فروق ذات دلالة إحصائية بين النساء اللواتي يعانين من متلازمة المبيض متعدد الاكياس البدينات وغير البدينات في المعلمات المدروسة للهرمونات،العناصر والفلزات الثقيلة.

الكلمات الرئيسية: متلازمة المبيض متعدد الاكياس ، العناصر النزرة ، المعادن الثقيلة، العناصر الرئيسية، العقم .

Introduction

Citsycylop ovary syndrome (PCOS) is symptomatic nearly 6% to 10% of women of reproductive age (12–45 years old) [(1)]. It is a principal cause of female subfertility and the most frequent endocrine problem in women of reproductive age, although up to 70% of women with PCOS may be undiagnosed [(2)].

Polycystic ovary syndrome is characterized by anovulation, insulin resistance and hyperandrogenism. Anovulation causes irregular menstruation, amenorrhea, ovulation-related infertility and polycystic ovaries. Hyperandrogenism results in acne and hirsutism. Insulin resistance is often correlated with obesity, Type 2 diabetes, and high cholesterol level [(3)].

The etiology of PCOS is not yet entirely known. It is believed that it is a complex, multifaceted disease involving abnormalities in the hypothalamic-pituitary axis, uncontrolled ovarian steroidogenesis, excessive oxidative stress, aberrant insulin signaling and genetic/environmental factors [(4,5)].

Many studies have indicated increased oxidative stress in the patients with PCOS [(6,7)]. Several characteristics and associations of PCOS, including androgen excess, abdominal adiposity, insulin resistance and obesity, may contribute to the development of local and systemic oxidative stress which may reciprocally worsen these metabolic abnormalities [(8)].

The whole blood of heavy metals and serum levels of trace elements were altered in patients with PCOS [(9)]. Environmentally relevant levels of metals are also associated with modest changes in reproductive hormone levels [(10)].

Cadmium is a heavy metal and may have a role in the formation of reactive oxygen species (ROS) [(11)]. Lead may also lead to the production of ROS by depleting glutathione (GSH) and protein-bound sulfhydryl groups and enhancing lipid peroxidation [(12)].

Calcium is an essential macroelement to body organization and structure and is the material of many indispensable substances, such as 25-hydroxyvitamin D (25(OH)D) and high-density lipoprotein (HDL) cholesterol, which contribute to the cardiovascular system [(13)].

Magnesium, one of the abundant cations in the human body categorized as macro element, participates in enzymatic reactions and insulin secretion. Magnesium is closely related to diabetes mellitus type 2 and other metabolic diseases [(14)]. Magnesium deficiency is believed to indirectly enhance the oxidative damage of biomolecules by inducing a stress response. It is possible that a magnesium deficiency stimulates catecholamine release from the adrenal glands. However, catecholamine increases the production of ROS. This creates a vicious positive feedback cycle where, for example, elevated blood epinephrine levels result in a further reduction of the magnesium concentration. Contrastingly, Magnesium deficiency leads to the activation of the renin-angiotensin system that also induces oxidative stress [(15)].

Manganese is an essential micronutrient incorporated into many metalloenzymes and proteins involved in cell metabolism and regulatory pathways controlling oxidative stress [(16)].

Subjects and Methods

This study was carried out at Kamal Al-Samarrai Hospital (Center for Infertility treatment and *in vitro* Fertilization "IVF"), from October /2016 to April/2017.

Eighty-two women were enrolled in the study. Fifty-four of them were patients with PCOS, with the age (obese 29.04± 5.64, non-obese 26.56±4.97) and twenty-eight apparently healthy women were selected as controls, their age (obese 32.07±5.86 and non-obese 24.64±4.25). Every participant woman was interviewed and requested to answer a specially designed questioning format

including; demographic data, menstrual, obstetric, medical and family histories.

The diagnosis of PCOS in this study was 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 (polycystic ovary 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) [17]. With the exclusion of other etiologies (androgen-secreting tumors, thyroid disorder, Cushing syndrome, hyperprolactinemia, congenital adrenal hyperplasia). All of the patients were selected under the supervision of a specialist gynecologist. The studied women were divided into:

A- Fifty-four PCOS patients, they were subdivided into two subgroups based on their body mass index (BMI), which was calculated as the ratio of the body weight in kilograms to the square of its height in meters [18]. The two subgroups were as follows; twenty-seven obese PCOS patients with BMI ≥ 30 kg/m^2 (34.81±0.820), and another twenty-seven non-obese PCOS patients with BMI <30 kg/m^2 (26.41± 0.401).

B- Twenty-eight apparently healthy control women were subdivided into two subgroups; fourteen obese control subjects with BMI ≥ 30kg/m^2 (32.81±0.720), and fourteen non-obese control subjects with BMI <30 kg/m^2 (23.90±0.678).

Venous blood samples (10cc) was withdrawn from eachwoman between days two and four of menstrual cycle after overnight fasting. Blood samples were divided into two parts, one part (2.5cc) were collected in an anticoagulant containing tubes for direct measurement of lead (Pb), and cadmium (Cd) blood levels and the other part (7.5cc) were collected in gel and clot activator tubes without anticoagulant then samples left for 30 minutes to clot. After complete clotting, the serum was separated by centrifugation (centrifuged for 10 minutes at 3500 to 4000 rpm to obtain serum).

The collected serum was divided in 10 Eppendorf tubes [9 tubes kept frozen (-80˚C) until their assay, and the rest of them used for immediate measurement of fasting glucose level].

Assay

The whole blood levels of cadmium, lead and serum levels of magnesium, calcium, manganese were measured by atomic absorption spectrophotometer in Poisoning Consultation Center in Medical City-Baghdad. Serum luteinizing hormone (LH), follicle stimulating hormone (FSH) and total testosterone (TES) were tested by Enzyme-linked final fluorescent assay (ELFA) [19,20]. Serum prolactin (PRL) and serum thyroid stimulating hormone (TSH) were measured by a two-site sandwich immunoassay using direct chemiluminometric technology [21,22]. Fasting blood glucose was evaluated according to the method of Barham and Trindoer [23].

Statistical analysis

The results were expressed as a mean ± standard error of the mean (SEM) or percent changes. LEVENE test was used to test the homogeneity of variances for equality of variances of the several independent groups. One-way analysis of variance (ANOVA) and least significant tests were used for comparisons between the studied groups, and to examine the degree of significance. Pearson's correlation coefficient (r) was used to study the correlation of trace element (manganese), some macroelements and heavy metals with the measured parameters of hormones. The statistical analysis was performed using SPSS, version 16 [24].

Results

The anthropometric and demographic characteristics of Polycystic Ovary Syndrome (PCOS) patients and controls are summarized in table 1. As illustrated in table 2, the serum FSH level was significantly lower in obese PCOS patients than in the obese and non-obese control groups (P<0.01, P<0.05 respectively); there was no significant difference between obese and non-obese PCOS (p>0.05). Serum LH, LH/FSH ratio, prolactin and total testosterone levels were significantly higher in both obese and non-obese PCOS patients as compared to their corresponding control groups. Yet, there was no significant difference between obese and non-obese PCOS groups as well as between the control groups. TSH levels were significantly higher in the obese PCOS than in the obese control (P<0.05). Serum glucose levels were significantly higher in the obese PCOS than in the obese and non-obese control groups (P<0.05, P<0.01 respectively), but there was no significant difference between the non-obese PCOS and the non-obese control (P> 0.05). Table 3 showed that, the whole blood concentrations of lead (Pb) and cadmium (Cd) were significantly higher in the PCOS groups than in the control groups; yet, there was no significant difference between the obese and non-obese PCOS groups (P> 0.05), as well as between the obese and non-obese control

groups. Finally, serum concentrations of magnesium(Mg), calcium(Ca) and manganese (Mn) were significantly lower in both of the obese and non-obese PCOS groups as compared to their corresponding control groups; and there was no significant difference between the obese and non- obese PCOS group.

Table (1): The anthropometric and demographic Data of PCOS patients and controls

Groups / Variables	PCOS		Controls		P-value
	obese (27)	non-obese (27)	obese (14)	non-obese (14)	
Age(years)	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 ± 0.02	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%)	١٤ (100%)	14 (100%)	1.000

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

Table (2): Descriptive statistics of studied sex hormones with comparative significant studies between groups (A) and groups (B) in serum levels of sex hormones.

Variables	Groups Numbers	Mean ±SE	Groups(A)	Groups(B)	P-value*
FSH (mIU/ml)	Obese PCOS (27)	4.592 ± 0.194	Obese PCOS	Obese Control	0.004 (HS)
				Non-Obese PCOS	0.348(NS)
	Obese control (14)	6.499 ± 0.790		Non-Obese Control	0.030 (S)
	Non-obese PCOS (27)	5.092 ± 0.332	Obese Control	Non-Obese PCOS	0.031(S)
	Non-obese Control (14)	6.010 ± 0.657		Non-Obese Control	1.508 (NS)
			Non-Obese PCOS	Non-Obese Control	0.156 (NS)
LH (mIU/ml)	Obese PCOS (27)	5.006 ± 0.389	Obese PCOS	Obese Control	0.001 (HS)
				Non-Obese PCOS	1.000 (NS)
	Obese control (14)	2.840 ± 0.315		Non-Obese Control	0.000 (HS)
	Non-obese PCOS (27)	5.000 ± 0.428	Obese Control	Non-Obese PCOS	0.001(HS)
	Non-obese Control (14)	2.541 ± 0.259		Non-Obese Control	0.882 (NS)
			Non-Obese PCOS	Non-Obese Control	0.000 (HS)

Continued table (2)

Variables	Groups Numbers	Mean ±SE	Groups(A)	Groups(B)	P-value*
LH/FSH Ratio	Obese PCOS (27)	1.152 ± 0.114	Obese PCOS	Obese Control	0.000 (HS)
				Non-Obese PCOS	0.985 (NS)
	Obese control (14)	0.448 ± 0.068		Non-Obese Control	0.000 (HS)
	Non-obese PCOS (27)	1.095 ± 0.112	Obese Control	Non-Obese PCOS	0.000 (HS)
	Non-obese Control (14)	0.465 ± 0.055		Non-Obese Control	0.993 (NS)
			Non-Obese PCOS	Non-Obese Control	0.000 (HS)
Prolactin (ng/ml)	Obese PCOS (27)	27.54 ± 2.44	Obese PCOS	Obese Control	0.000 (HS)
				Non-Obese PCOS	0.796(NS)
	Obese control (14)	11.30 ± 2.02		Non-Obese Control	0.000 (HS)
	Non-obese PCOS (27)	24.06 ± 2.91	Obese Control	Non-Obese PCOS	0.005 (HS)
	Non-obese Control (14)	9.99 ± 1.61		Non-Obese Control	0.956(NS)
			Non-Obese PCOS	Non-Obese Control	0.001 (HS)
Total testosterone (ng/ml)	Obese PCOS (27)	3.078 ± 0.363	Obese PCOS	Obese Control	0.000 (HS)
				Non-Obese PCOS	0.882(NS)
	Obese control (14)	1.229 ± 0.104		Non-Obese Control	0.000(HS)
	Non-obese PCOS (27)	2.667 ± 0.424	Obese Control	Non-Obese PCOS	0.031(S)
	Non-obese Control (14)	1.175 ± 0.163		Non-Obese Control	0.992 (NS)
			Non-Obese PCOS	Non-Obese Control	0.012 (S)
TSH (mIU/L)	Obese PCOS (27)	3.064 ± 0.868	Obese PCOS	Obese Control	0.018 (S)
				Non-Obese PCOS	0.054(NS)
	Obese control (14)	0.935 ± 0.109		Non-Obese Control	0.075 (NS)
	Non-obese PCOS (27)	1.639 ± 0.156	Obese Control	Non-Obese PCOS	0.428 (NS)
	Non-obese Control (14)	1.472± 0.259		Non-Obese Control	0.598 (NS)
			Non-Obese PCOS	Non-Obese Control	0.850 (NS)
FSG (mmol/l)	Obese PCOS (27)	6.530 ± 0.387	Obese PCOS	Obese Control	0.012 (S)
				Non-Obese PCOS	0.122(NS)
	Obese control (14)	5.229 ± 0.149		Non-Obese Control	0.002 (HS)
	Non-obese PCOS (27)	5.874 ± 0.312	Obese Control	Non-Obese PCOS	0.207 (NS)
	Non-obese Control (14)	4.936± 0.193		Non-Obese Control	0.616 (NS)
			Non-Obese PCOS	Non-Obese Control	0.068 (NS)

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

Table (3): Descriptive statistics of trace elements and heavy metals with significant comparative studies between groups (A) and groups (B) in blood levels of studied elements

Variables	Groups	Mean ±SE	Groups(A)	Groups(B)	P-value*
Lead (µg/dl)	Obese PCOS (27)	22.48 ± 0.42	Obese PCOS	Obese Control	0.000 (HS)
				Non-Obese PCOS	0.360 (NS)
	Obese control (14)	14.29 ± 0.42		Non-Obese Control	0.000 (HS)
	Non-obese PCOS (27)	23.00 ± 0.45	Obese Control	Non-Obese Morbid	0.000 (HS)
	Non-obese Control (14)	14.29 ± 0.44		Non-Obese Control	1.000 (NS)
			Non-Obese PCOS	Non-Obese Control	0.000 (HS)
Cadmium (µg/dl)	Obese PCOS (27)	0.317 ± 0.009	Obese PCOS	Obese Control	0.000 (HS)
				Non-Obese PCOS	1.000 (NS)
	Obese control (14)	0.144 ± 0.006		Non-Obese Control	0.000 (HS)
	Non-obese PCOS (27)	0.318 ± 0.011	Obese Control	Non-Obese PCOS	0.000 (HS)
	Non-obese Control (14)	0.139 ± 0.008		Non-Obese Control	0.974 (NS)
			Non-Obese PCOS	Non-Obese Control	0.000 (HS)
Magnesium (mg/dl)	Obese PCOS (27)	0.979 ± 0.025	Obese PCOS	Obese Control	0.000 (HS)
				Non-Obese PCOS	0.721 (NS)
	Obese control (14)	1.410 ± 0.038		Non-Obese Control	0.000 (HS)
	Non-obese PCOS (27)	0.963 ± 0.030	Obese Control	Non-Obese PCOS	0.000 (HS)
	Non-obese Control (14)	1.378 ± 0.060		Non-Obese Control	0.706 (NS)
			Non-Obese PCOS	Non-Obese Control	0.000 (HS)
Calcium (mg/dl)	Obese PCOS (27)	7.319 ± 0.111	Obese PCOS	Obese Control	0.000 (HS)
				Non-Obese PCOS	0.228(NS)
	Obese control (14)	9.107 ± 0.174		Non-Obese Control	0.000 (HS)
	Non-obese PCOS (27)	7.082 ± 0.153	Obese Control	Non-Obese PCOS	0.000 (HS)
	Non-obese Control (14)	8.979 ± 0.228		Non-Obese Control	0.6 (NS)
			Non-Obese PCOS	Non-Obese Control	0.000 (HS)

Continued table (3)

Variables	Groups	Mean ±SE	Groups(A)	Groups(B)	P-value*
Manganese (µg/dl)	Obese PCOS (27)	0.086 ± 0.002	Obese PCOS	Obese Control	0.000 (HS)
				Non-Obese PCOS	0.931(NS)
	Obese control (14)	0.225 ± 0.009		Non-Obese Control	0.000 (HS)
	Non-obese PCOS (27)	0.084 ± 0.002	Obese Control	Non-Obese PCOS	0.000 (HS)
	Non-obese Control (14)	0.236 ± 0.009		Non-Obese Control	0.837 (NS)
			Non-Obese PCOS	Non-Obese Control	0.000 (HS)

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

Correlation studies

Pearson´s correlation was conducted to study the association of the alterations in the levels of the measured elements, with some of the hormonal changes occurring in PCOS. The results are shown in Table 4.

Table (4): Pearson´s correlation of the measured elements and heavy metals with the hormone levels in the obese PCOS and non-obese PCOS groups

Groups	Hormones	Correlation and Significant	Elements and heavy metals				
			Pb	Cd	Mg	Ca	Mn
Non-obese PCOS	FSH	r	-0.145	0.271	-0.180	0.089	0.093
		p	0.471	0.171	0.368	0.660	0.643
	LH	r	0.344	0.066	-0.43*	-0.012	0.267
		p	0.079	0.742	0.027	0.954	0.642
	LH\FSH	r	0.366	-0.202	-0.078	-0.065	0.310
		p	0.061	0.313	0.698	0.748	0.116
	TSH	r	0.384*	-0.138	0.262	-0.028	0.245
		p	0.048	0.494	0.187	0.891	0.218
	Prolactin	r	-0.005	-0.202	0.053	0.066	-0.080
		p	0.981	0.312	0.846	0.744	0.690
	Testosterone	r	0.109	0.064	0.203	0.168	0.045
		p	0.589	0.751	0.309	0.402	0.824
	FBS	r	0.061	0.280	-0.141	0.057	-0.070
		p	0.761	0.157	0.482	0.780	0.728
Obese PCOS	FSH	r	0.084	-0.118	0.141	-0.233	-0.130
		p	0.677	0.559	0.483	0.241	0.518
	LH	r	0.012	-0.063	0.107	-0.014	0.202
		p	0.953	0.756	0.597	0.945	0.313
	LH\FSH	r	-0.009	-0.046	0.008	0.152	0.171
		p	0.966	0.821	0.970	0.448	0.393
	TSH	r	0.122	-0.130	-0.143	-0.044	0.090
		p	0.544	0.519	0.478	0.826	0.654
	Prolactin	r	0.254	0.090	0.023	0.632	-0.371
		p	0.201	0.657	0.908	0.743	-0.130
	Testosterone	r	-0.070	0.465*	-0.123	0.330	0.004
		p	0.727	0.015	0.540	0.093	0.986
	FBS	r	-0.207	-0.260	0.212	-0.060	0.078
		p	0.299	0.191	0.288	0.767	0.699

*Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

There was positive correlation between blood lead and serum TSH levels (r 0.384, p 0.048) in non-obese PCOS women (figure 1). In addition, there was positive correlation between serum total testosterone and cadmium levels (r 0.465, p 0.015) in Obese PCOS Women (figure 2). Furthermore, there was negative correlation between serum magnesium and serum LH levels (r - 0.43, p 0.027) in non-obese PCOS women (figure 3).

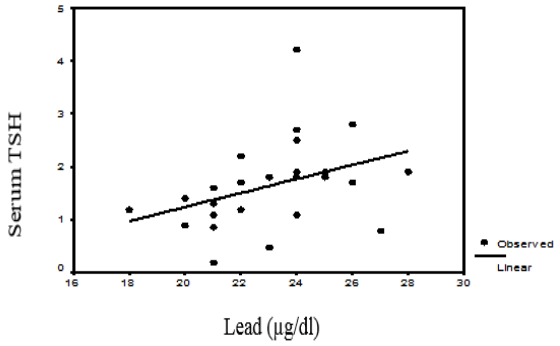

Figure (1): Correlation between blood Lead (µg/dl) and serum TSH (mIU /L) (r 0.384, p 0.048) in non-obese PCOS women.

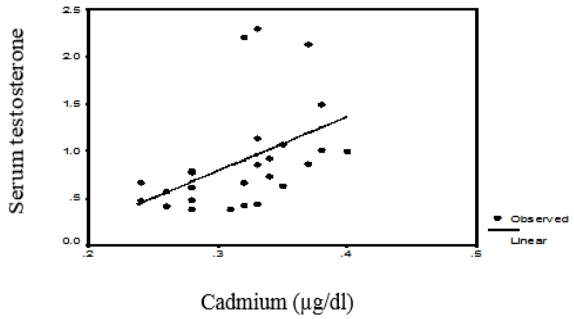

Figure (2): Correlation between Serum total testosterone (ng/ml) and Cadmium(Cd) (µg/dl) (r 0.465, p 0.015) in Obese PCOS Women.

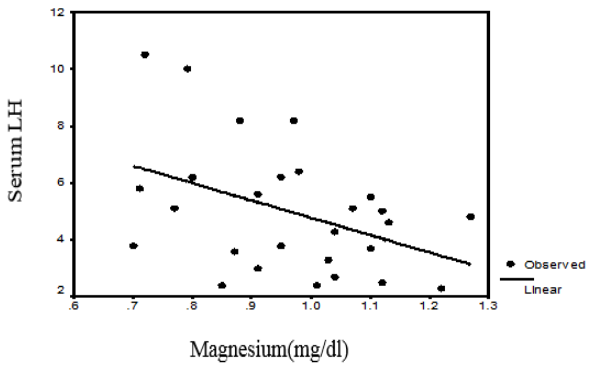

Figure (3): Correlation between serum magnesium (mg/dl) and serum LH (mIU /ml) (r - 0.425, p 0.027) in non-obese PCOS women.

Discussion

The diagnosis of PCOS in this study was based on Rotterdam criteria that are confirmed by the results obtained from analysis data related to the measured hormones [25]. Trace elements and heavy metals might be involved in the development of PCOS [9]. The present study was designed to investigate serum heavy metal, manganese and some macroelement concentrations in relation to hormone levels in PCOS. In table 3, lead (Pb) levels were significantly higher in PCOS patients than in the control groups. And there was a positive correlation between blood lead concentration and serum TSH level in non-obese PCOS patients (r 0.384, p 0.048). This occurs in accordance with Singh *et al*. who has declared that, an increase in serum TSH levels was associated with exposure to lead, without alteration in serum T3 and T4; and proposed that lead may enhance pituitary TSH release [26]. Chang *et al*. has investigated the relationship between lead exposure and the risk of infertility in Taiwan women, reporting mean blood lead levels of 35.5 and 27.8 µg/L in infertile women and pregnant women, respectively. The blood lead level was significantly higher in infertile women [27]. The pathogenic effect of lead is multifactorial since it directly hinders the activity of enzymes, competitively inhibits absorption of important trace minerals and deactivates antioxidant sulfhydryl pools. Free radical-induced damage by lead is accomplished by two independent, although related mechanisms. The first involves the direct formation of ROS including singlet oxygen, hydrogen peroxides, and hydro peroxides, and the second mechanism is achieved by depletion of the cellular antioxidant pool. Interrelations between these two mechanisms exist so that the increase in ROS on one side simultaneously leads to depletion of antioxidant pools on the other [28].
Table 3 also showed that, there was significantly higher blood concentration of cadmium in PCOS patients than in controls, and there was a positive correlation between blood concentration of cadmium and serum total testosterone levels, which is one of the main characteristics of PCOS, in obese PCOS patients (r 0.465, p 0.015). The toxic mechanisms of cadmium are not well understood, but it is known to act intracellularly, mainly via free radical-induced damage, particularly to reproductive organs [28]. As with lead, the higher cadmium levels in PCOS may contributes to the creation of ROS. The possible mechanism explaining the role of cadmium in free radical generation may involve displacement of copper and iron by

cadmium from binding protein, the displaced copper can catalyze breakdown of hydrogen peroxide via the Fenton reaction [28]. Excessive generation of ROS is documented in patients with PCOS [6]. Therefore, lead (Pb) and cadmium (Cd) may have a significant role in the pathogenesis of PCOS linked to the oxidative stress.

Calcium, is a basic element in the human skeletal system, maintains body growth and is involved in muscle activity and neurotransmitter release. Both of the obese and non-obese PCOS patients in this study, had significantly lower levels of serum calcium than in the control subjects as can be seen in the table 3. Similar findings were shown by Li M *et al* [29]. Another study has showed that serum calcium levels are low in PCOS patients, and these low levels can cause atherosclerosis [30]. Mazloomi *et al*. has reported that calcium positively affects PCOS [31]. In addition, a clinical study has found that calcium and vitamin D supplements are effective medicines for PCOS [32].

Concerning magnesium, the results indicated a significantly lower levels, in both of the obese and non-obese PCOS patients, than the levels in the control groups, as can be seen in table 3. Usharani *et al.* has revealed a lower level of serum magnesium in PCOS patients [33]. Hypomagnesemia alters glucose entry into the cells leading to insulin resistance, thus, dysregulation of the hypothalamus-pituitary-gonadal axis [34]. The serum LH level was inversely correlated with magnesium levels in non-obese PCOS patients. Fantidis *et al.* agrees with our result, by showing that low magnesium level in rats was associated with increased release of LH hormone [34].

Table 3 showed that, serum manganese levels in both of the obese and non-obese PCOS patients, were significantly lower than that in the corresponding control subjects. Manganese is a cofactor for some metalloenzymes, like manganese-containing superoxide dismutase SOD (MnSOD). It neutralizes the highly reactive superoxide ions to less reactive hydrogen peroxide (H_2O_2), which is followed by its immediate conversion to H_2O by catalase and other peroxidases in the mitochondrial matrix, hence, it protects against oxidative stress [35]. The result of the present study regarding manganese occurs in accordance with another study, that showed lowered serum level of manganese in PCOS patients as compared to that of control subjects [36].

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